"BANKED BLOOD"

A Study in Blood Preservation

by

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Submitted in partial fulfillment of the
requirements for the degree of Doctor of
Medical Science in the Faculty of Medicine,
Columbia University.

ACKNOWLEDGMENTS

The thought, labor, advice and monetary assistance of many individuals and groups have made this work possible. I am particularly grateful to:

Dr. Allen O. Whipple, for the privilege of working in the Surgery Department and the pleasure of so many fine associations.

Dr. John Scudder, who suggested this investigation and has actively participated in or guided nearly every step of it.

Dr. A. Purdy Stout, for the many courtesies extended to me while working in the Department of Surgical Pathology.

Dr. Hans T. Clarke, for valuable instruction in some of the difficult spots.

Mrs. Ethel B. Gutman, for instruction and use of her methods in the magnesium and phosphorous determinations.

Dr. B. Padersky of Palestine, now a Research Fellow at the Mt. Sinai Hospital, for translations of over twenty of the most important Russian works on blood transfusions; likewise, to Mr. and Mrs. John Ivanoff and Miss Olga Mordwin,

for great aid in translating other Russian articles and checking the Russian bibliography.

All of my associates in the laboratory throughout the period of study, in particular, Miss Dorothy Corcoran, Miss Eunice Thompson, Mrs. Margaret Nelson, Mrs. Johnette Barnock and Miss Ruth Liebhold. To Dr. Margaret Smith goes the entire credit for working out the technique of the ammonia determinations and to Miss Elizabeth Tuthill, co-author of the sodium method, especial thanks for bearing the brunt of most of the later chemical determinations.

Miss Helen Stoddard, indefatiguable nurse in charge of the "blood bank", and Mr. Josiah Lasell, hematologist.

The General Education Board of the Rockefeller Foundation, for the Fellowship which made this period of study possible.

The Blood Betterment Association of New York City, for funds to carry out much of the experimental work.

Miss Mary W. Sargent, without whose constant aid in every phase of its preparation this work would not have been possible.

TABLE OF CONTENTS

TABLES

ILLUSTRATIONS

"BANKED BLOOD"

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CHAPTER I

INTRODUCTION

The transfusion of blood from one individual to another is still a potentially dangerous operation.

In spite of the very marked advances in the knowledge of physiology, bacteriology, immunology, and the physical chemistry of biologic processes as applied to the practice of transfusions, deaths still occur following transfusions and no clinic has been able entirely to free the procedure from unfavorable reactions in the form of chills, fever, urticaria, jaundice, hemoglobinuria and other nonfatal but very disturbing phenomena.

Since 1937 the practice of storing blood over periods of days or weeks has grown very rapidly in the country. The common depot in a hospital to which donors are sent to give or deposit blood in anticipation of later need, and from which withdrawals can be made at a later date, was called by Fantus (1937) a "blood bank". At present there is a rapidly growing

number of setups of this character.

The advantages of having on hand at all times bloods of each group are obvious, especially in case of dire emergencies when there is insufficient time to get voluntary or professional donors. Certain disadvantages, however, immediately come to mind: the blood is older; changes may be taking place which make such blood less desirable, even undesirable. The questions which naturally arise are: Is such blood dangerous? Is it capable of normal function when used for transfusion? Are the advantages of speed and utility in its use offset by decreased therapeutic value for the patient? Are reactions increased or decreased?

This series of studies was begun in an attempt to answer the first question: Is blood which has been kept in a preservative of some kind for varying periods of time dangerous? If so, in what quantities and in what conditions? Finally, what is the toxic factor?

Scudder, Zwemer, Truszkowski, and Whipple in a series of publications (356, 357, 358) from this institution pointed out the role of potassium in the "toxemias" associated with intestinal obstruction, severe dehydration, shock, and trauma. The essence of their finding is that when tissues are destroyed or injured, tissue juices are thrown into circulation. These juices are rich in ions normally present only in the cells and

when present in increased amount in the plasma become extremely toxic to the organism as a whole. The chief among these mineral bases of the cells is potassium. If this is a toxic substance, it is of interest to know how much of it is freed from the cells by the injury incident to phlebotomy and continuing as a result of preservation in various solutions which themselves in excess are injurious to living tissue.

The first experiment in the present series was set up to determine how much of this cation is released from the cells.

Having shown that it is released in increasingly great amounts as the blood grows older and that this change is hastened by trauma, it next became necessary to establish if possible some measuring rod for quickly assessing the amount of change. Since the plasma of fresh blood is clear amber colored and that of old blood a marked red, it was felt that perhaps the degree of hemolysis would be a fair index of cell destruction. To test this, the second series of observations were made.

In the natural sequence of events, it became necessary to know just how toxic potassium is. The animal experiments were done in an attempt to answer this question.

Then the question arose: Are these changes indicative

of true destruction of the cellular elements or may not changes in the permeability of the cells or shift in concentration gradients account for the findings? To establish this point, studies were carried out with the idea of determining the fate of the cellular elements.

The study on bloods at death was an attempt to correlate experimental and clinical data.

Since the idea of cadaver and placental bloods for transfusions has gained a number of advocates (64, 143, 363, 411), a study of potassium changes in them seemed to offer a common basis of comparison with fresh and stored bloods from healthy adult donors.

When it was observed that the rate of cell breakdown in cadaver blood as measured by the rate of potassium diffusion was markedly greater than that in the donor blood, the inevitable question arose. Why? Jacques (1938) showed that if the concentration of ammonia in sea water is raised by 0.0001 molar, there occurs a rapid exit of potassium from the marine alga, Valonia macrophysa, Kütz. The cell water of the human erythrocyte is quite comparable in ionic content to that of the Valonia, just as the sea water in which it lives has a compositon very similar to that of blood plasma. If the addition of ammonia to sea water causes increased rate of loss of potassium from the sea cell, may not the autolysis of protein in the cadaver liberate sufficient ammonia to

account for the increased diffusion of red cell ions? It became of interest, therefore, to determine just how much ammonia cadaver blood did contain and, carrying the investigation one step further, to see if the rate of diffusion in normal blood to which ammonia had been added approached that of cadaver blood. One other factor made it necessary to determine the ammonia content of the cadaver blood before the potassium values could be accepted as valid. It is well known that with the argenticobaltinitrite method used in these experiments (232, 388) any ammonia present will be quantitatively precipitated as ammonium cobaltinitrite along with the potassium salt and thereby give false high readings of the latter.

This work with ammonia led to a reconsideration of the observation previously noted that hemolysis was less marked in the bloods whose pH values approached or went below 7.0 toward the acid side. To determine whether or not this was simply a coincidence or a genetically related phenomenon, further studies were undertaken to explore the constancy of this association.

Up to this point the chief interest had been centered in the potassium changes in preserved blood, but its changes could give at best only a partial picture of the process.

When the ionic equilibrium of body fluids is disturbed by marked changes in concentration or position of some one element, there is immediately brought into play certain compensatory mechanisms on the part of the organism as a whole in an effort to maintain acid-base balance (124, 134, 164, 266). These dictate changes in concentration and location of other elements.

With such marked shifts in the potassium content of cells and plasma, an attempt was made to trace the shifts in other cations and anions with the hope that in so doing a method would suggest itself for more completely stabilizing all of the elements.

In these times of war when men die on many fronts, it becomes imperative to get either fresh or well preserved blood to them or some substitute capable of sustaining osmotic balance and circulation in the early hours of their injury. It is difficult with present methods to supply whole blood for soldiers far removed from large centers of civilian population in spite of excellent systems of supply such as that devised by Duran Jordá (202) in the Spanish war. Plasma, however, may be kept for long periods (257, 380) without marked changes in protein content or oncotic power. The pressing needs for further knowledge of its qualities and short comings is sufficient justification for the studies just begun in this direction.

We had, it was felt, at the conclusion of these investigations and theoretical considerations certain grounds for suggesting that while preserved blood had many points to recommend it, there are limitations to its usefulness and a few definite contraindications.

To test these views it became necessary to observe the course of patients following transfusions of blood of various ages, preserved in different anticoagulants, stored in different types of containers, and given by different methods.

The establishment of the experimental blood bank in Presbyterian Hospital in the summer of 1939, fortunately, presented the opportunity for making these observations.

Transfusions are given so routinely and with so little excitement now that one is likely to overlook the fascinating but hectic history of the operation.

Two names stand above all the rest from among those who have played a part in raising the practice of blood transfusion from the realms of mythology to the status of an accredited type of therapy in modern scientific medicine. The first is that of Harvey who, in 1616, discovered the circulation of the blood and so stimulated medical thought with his lectures and his monograph, published in 1628, that investigators in many lands began to experiment with the infusion of various substances into the blood stream and to seriously consider the replacement of old blood by new (271). For nearly three

centuries little true progress was made because the source of danger in blood transfusions was not clearly understood.

The second of the two great names is that of Landsteiner who, in 1900, showed that the serum of one normal human being can destroy the blood cells of another individual. Clumping had been previously observed by Creite (86) and Landois (237), and in 1899 Shattock independently published observations somewhat comparable to Landsteiner's; but it is to the latter that the credit must go for it was he who fully realized the practical significance of his findings and pointed out the importance of the blood groups for blood transfusions in his original paper.

Using these two epochal advances as landmarks, the historical study of transfusions divides itself into three periods:

1. Before Harvey, which may be called the period of its infancy.
2. Between the publication of Harvey's monograph and Landsteiner's original paper, which may be considered the adolescent period of its development.
3. Since Landsteiner's paper in 1900, which may be considered as the period of its maturity.

Each of the decades of this mature period has been marked by a dominant theme: the first, by improvements in methods

of direct transfusion; the second, by the search for means of making indirect transfusions more feasible; and the third, by attempts to preserve blood for purposes of delayed transfusion or use at distances relatively far removed from the place of its reception from the donor. Following the analogy to its natural conclusion a fourth period, that of senescence, must be contemplated should the many attempts now being made to replace blood as a therapeutic agent reach fruition in the production of some ideal synthetic substitute.

CHAPTER II

EVOLUTION OF THE BLOOD BANK

Part I

Before Harvey

(1) Mythology

The history of blood transfusion begins, as most history does, in the mythology of the ancient peoples. No records exist of actual transfusion in these earliest times, but the idea of restoring the youth and spirit of the old and dying by the injection of the blood of the strong had its birth before the recorded memory of man (323). It could reach maturity only as the result of certain scientific and technical advances which have slowly taken place. The "banking" of blood is the latest of these advances. It is with this technique that we are chiefly concerned, but it seems wise to explore first some of the more important earlier steps in the development of this idea.

Ovid (307) reports that Jason implored his wife, a sorceress, to restore youth to his aged father, Aeson, for the festivities attending the return of the Argonauts from Colchis. Accordingly, "Medea unsheathed her knife and cut the old man's throat, then, letting the old blood run out

she filled his veins with brew so potent that a withered olive branch with which she stirred it at once bore leaves and fruit. When Aeson had drunk this, in part through his lips and in part through his wound, his beard and his hair lost their hoary grey and quickly became black again; went the pallor and the look of neglect; the deep wrinkles were filled out with new flesh; his limbs had the strength of youth and Aeson was filled with wonder."

Even today such miracles are expected from simple infusions and small transfusions when the rationale of their exhibition is as miasmic as that of the ancient Media.

(2) Humoral Spirits

In the earliest periods blood was sought not so much for its value in restoring measurable physical properties but rather for its metaphysical attributes. According to the teaching of Galen (62), the blood and the natural spirit arose from the liver and was carried to the heart which was the seat of the vital spirit from whence it was sent to the brain to be perfected and become the animal spirit. For a complete soul these three elements had to be present and it was to restore these elements, the epithumos, the thumos, and the hegemonos, that the blood of young warriors was recommended as a draught (323).

(3) Early Transfusions

The first record of a transfusion being given in a manner comparable to today's methods is recordin in Pasquale Villari's "Life and Times of Girolamo Savonarola" as follows: "--the vital powers of Innocent VIII were rapidly sinking. He had been lying for some time in a lethargic state, that was occasionally so death-like as to make his attendants believe that all was over. Every means of restoring his exhausted vitality had been tried in vain, when a doctor proposed to attempt to cure by means of a new instrument for the transfusion of blood. Hitherto this experiment had only been tried on animals; but now the blood of the decrepit Pontiff was to be transfused into the veins of a youth, who gave his own in exchange. Thrice, in fact, was the difficult experiment made. It did no good to the Pope; and the three boys, costing the sum of one ducat apiece, lost their lives, through the introduction of air into their veins. The doctor then fled, and on July 25, 1492 (five days later), Innocent VIII expired." (398).

Scheel (1802) tells us that Hieronymus Cardanus (1576) and Magnus Pegelius (1593) discussed the possibilities of direct transfusions while Andrius Libavius of Coburg in 1615 described in great detail a method for direct transfusions which became the most successful type used in the

early part of the present century. Professor Johannes Colle of Padua very plainly stated in 1628 that the giving of blood from a young man into the veins of an old man would seem good therapy. Later in this same year Harvey's monograph (165) on the circulation of the blood appeared, but it is well to remember that he had been lecturing on what later became his book for fifteen years before its publication. Treatises came out for many years to show that this was not an original observation, but his place in the history of medicine seems secure.

CHAPTER II

Part II

After Harvey and Before Landsteiner

(1) Intravenous Medication

The first actual record of an intravenous infusion, Ettmuller (1691) tells us, is found in the story of a certain Austrian nobleman and sportsman of about 1642 whose kennel master delighted in blowing wine through a quill into an open vein in one of his dogs. The vein then being tied, the inebriated howlings and actions of the animal would cause great merriment for the owner and his guests until the beast, exhausted with the involuntary bacchanalia, would collapse, dead drunk. Strange, is it not, that a master of the hounds should have given the first recorded intravenous infusion twenty years before the learned gentlemen of the world fought over the right of priority?

In higher circles it seems that the first suggestion that intravenous infusions might be plausible was made by Sir Christopher Wren (53), the astronomer and architect, in 1656. Out of the work of Boyle (52, 54) and his contemporaries, Coxe (85), Wilkins, and Lower (269), much progress was made in the early knowledge of the blood and the effects of drugs given intravenously. The first recorded treatment of man by intravenous medication seems to have been done by J. D. Major

in 1664 (62).

(2) Transfusion from Animal to Animal

The first transfusion from animal to animal was carried out by Richard Lower (270) of Oxford in 1665. The honor of having done the first transfusion was seriously contended by Regneri de Graef (148) who claimed the honor for a fellow Dutchman, Ludwig des Bils; by the Italian Franz Folli (281) who claimed that he had done a transfusion in 1654; and by Professor John D. Major of Kiel, in a publication in 1667, mentioned himself as the first to do a blood transfusion (62). The latter's methods sound very modern, but he was disclaimed as somewhat of a quack by the savants of his day.

(3) Transfusion from Animal to Man

There seems little doubt that Jean Baptiste Denis (sometimes spelled Dionis)(100), professor of philosophy and mathematics in the University of Paris, gave the first transfusion from animal to man in April, 1667. Being successful in this first lamb's blood transfusion, he went on to give many more and became very famous, but a death following his fourth transfusion caused such a storm of bitter discussion that he was brought before the court on a charge of murder. Though he was exonerated in 1668, a law was passed by the

French Parliment which specifically prohibited transfusion experiments on human beings in France. The magistrates of Rome were so impressed by the ruling of the French peers that they passed a similar decree. About 1678 the Pope passed a special edict banishing transfusions so that the practice was practically buried for one hundred fifty years (138).

(4) Transfusion from Man to Man

Paul Scheel of Copenhagen attempted to revive the practice in 1802 with a splendid compilation of that which had gone before, but the honor of bringing the discarded procedure back into prominence must go to James Blundell (43) of London. His is the merit of having first given a transfusion from man to man. He accredited his first notions on the subject to a Doctor Leacock of Barbados.

He specifically stated that "the blood of one genus of animals can not be indifferently substituted, in large quantities with impunity, for that of another genus; and, therefore, that if an operation be performed upon the human body, human blood only should be employed." His publication in 1824 pictured his "Impellor" which was the first of a long series of more or less complicated apparatus for use in transfusions. His syringe was quite modern in type and the conclusion "that transfusion by syringe is a very feasible

and useful operation" has been adequately borne out, but his second conclusion that the operation is not attended with obviously dangerous symptoms of course could not stand up with the state of knowledge as it was at that time. Dieffenbach (414), in 1828, commenting on Blundell's work, stressed the fact that blood preserved over three hours and kept liquid through shaking, when injected into the blood of another animal is promptly fatal.

(5) Defibrinated Blood Transfusions

Bischoff, in 1835, decided that the toxic agent in such blood was fibrin, therefore only defibrinated blood should be used. He felt that only the cells carried the true vivifying element and the serum and fibrin were unessential.

Larson, a student of Panum (309), in 1847, was the first to use defibrinated blood for transfusion. Brown-Séquard (1857) agreed with Bischoff that there was some toxic factor in blood and defibrinated bloods could be safely used providing the carbonic acid content was not too great. He felt that the source of the toxicity probably lay in the acid-base changes. Esmarch (114), the Prussian surgeon, gave defibrinated blood its greatest clinical trial in the Franco-Prussian war and was one of the first to condemn it.

Gesellius (138) called defibrinated blood just blood that had been "beaten to death" and spoke against its use. In spite of much good work showing the danger of its use, both before and after the discovery of the isoagglutinogens (170, 237, 289, 291, 320, 346), there are reports of its continued use by Opitz in 1924 and by Plehm, Denecke, and Platt in 1926.

(6) A Period of Reflection

Outstanding were the rather complete reviews of Oré (1868) of Bordeaux, von Belina-Swiontkowski (1869) of Heidelburg, Drinkard (1872) of Washington, D. C., Gesellius of St. Petersburg (1873), Landois of Greifswald (1875), and Roussel of Geneva (1876).

Oré (302) stressed the danger of introducing air into the veins, also the variations of toxicity with introduction of oxygen, hydrogen, and carbon dioxide. Amounts necessary for fatal issue seemed to be dependent on the individual solubilities of the gases in the blood. The following table is adapted from his work and shows the status of transfusions in 1868.

	Number of Cases	Cured	Improved	Unchanged	Dead
Animal blood	154	64	20	44	26
Human blood	381	185	15	5	178
	535	249	35	49	204

It is true that almost one half of the patients died, but death in most instances could not have been due directly to

the transfusion. The selection of cases was so poor, the risks so great that in the light of present knowledge it is surprising that so many lived.

Von Belina-Swiontkowski (28) cites one hundred fifty-five cases from the literature, goes into quite some detail in their analysis and recommends indirect transfusion with defibrinated blood. Drinkard (104) found one hundred seventy cases in the literature, states that up to that time there were only four cases on record in America and during the whole Civil War only one transfusion seems to have been given.

Gesellius (138) advocated the return to animals as the source of blood and may be said to have started a new, but small, wave of heterologous transfusions. Though his reasoning was sound and motives humanitarian, he advocated what seems to be a step backward. He was not, however, the last to advocate the use of animal blood for transfusion for Cruchet, Regot, and Caussimon (88) published a monograph in favor of the practice in 1928.

Landois (237), among other things, concerned himself with the physiology of the blood and the cause of febrile reactions. By many transfusions and a series of experiments he showed that the sera of one species of animals coagulated and dissolved the cells of other species; explained the hemoglobinuria following heterologous transfusions upon this basis;

stressed again that because of this great danger only human blood be used for humans; and because of the still unexplainable danger inherent even in homologous transfusions between men, that perhaps it was wisest to use intravenous saline. On the authority of this excellent work transfusion again waned, but Landois' name must rank high in the list of the students of the subject. He might well be considered the last of the men of the adolescent period.

CHAPTER II

Part III

After Landsteiner

(1) The Theory of Blood Groups

Creite in 1869 had observed the clumping of human red corpuscles when placed in the sera of cats, dogs, sheep, or birds; and Landois definitely established the fact that this coagulation need not be associated with any pathological process. He recognized agglutination as a physiological process. Bordet in 1895 had definitely established the fact that serological methods could be utilized for the differentiation of species differences in animal cells and proteins.

Landsteiner (240), knowing of the work of Bordet, postulated that if by such means species differences could be detected perhaps the same principle would be applicable to the detection of individual variations within the species. In his first series of experiments in 1900, he mixed red blood cells with the sera of other healthy human beings and got a result which was totally unlooked for. Where he had expected the appearance of perhaps minor agglutination phenomena, to his surprise some of the mixtures remained completely unchanged while in others clumping of the cells was as marked as though the bloods had been taken from a different

species of animals. He concluded in his first paper that not only is agglutination of the cells of an individual possible by serum of a different species; i.e., hetero-agglutination, but that in the same animal type there may occur the phenomenon of isoagglutination.

Soon after this Ehrlich and Morgenroth (1901) injected a series of goats with blood from other goats and demonstrated isoantibodies in the serum of these animals which were active against the cells of the same species. This was called iso-hemolysis. It added evidence that not only was there such a thing as species specificity but also specificity within a species.

In a second publication, Landsteiner (1901) demonstrated that these reactions did not occur at random but seemed to follow a very definite rule. Individuals, it seemed, could be grouped into three different types according to the reaction of their cells and serum with the cells and serum of other individuals. He found in group A the serum agglutinates the corpuscles of group B but not those of its own group. In group B, the serum agglutinates the cells of individuals of group A but not those of its own group. In group C, the serum agglutinates the corpuscles of both A and B but not those of group C.

He offered his theory of isoagglutination and called

attention to the importance of this observation in relation to therapy. To account for this group differentiation, he postulated the existence of two agglutinogens.

As a continuation of this investigation von Decastello and Sturli in 1902 found four individual exceptions to Landsteiner's three groups. Following the technique of Bordet, they demonstrated the presence of two agglutinins, one found in serum A, one in serum B, and both in C. No blood ever had an agglutinin in the serum, it appeared, which would act against its own cells. This work was repeated and confirmed by Hektoen in 1907 and was probably more widely read than either of the earlier publications. The exceptions to Landsteiner's rule seemed to occur in group C, but at this time the fourth distinct group was not definitely postulated as we know it today.

It has been felt by some (414) that perhaps Shattock should have shared some of the honor of these early days with Landsteiner for in an article (1899) which preceded the latters', the clumping of chromocytes was described in relation to certain disease processes. Noting the clumping of pneumococci in a hanging drop preparation and noticing the clumping of the red blood cells from the same patient, he postulated that there must be some element or some changes in the serum which caused the clumping in each case; and he concludes "and if the hypothosis of an increased amount of 'agglutinating' substance meets the first, it may be extended to the second."

Nowhere, however, does he relate his observation to the problem of blood incompatabilities in healthy individuals or distinguish between pseudoagglutination and true isoagglutination. His findings, viewed in the light of later investigation, yield more information than they did to the observer.

It was left for Jansky in 1907 to incorporate the exceptions noted by von Descastello and Sturli and Hektoen into a fourth group. His biological scheme as compared to Landsteiner's was as follows:

	Landsteiner	Jansky
Group	A	II
Group	B	III
Group	O	I
Group	Exceptions	IV

The characteristic of this fourth group was that its serum would not coagulate the cells of any of the other groups, but that its cells were coagulated by the serum of all the other groups. His work was published in a little-known journal and like the work which preceded him did not get widespread clinical application. In the book by Crile, "Hemorrhage and Transfusions," published in 1909, which was certainly the best of its time and did much to stimulate anew the question of transfusions, Jansky is not mentioned; while Hektoen is quoted as follows: "due to the occurence of isoagglutinins in human blood, under special conditions, homologous transfusion might prove dangerous by erythrocytic

agglutinations within the vessels of the subject transfused," Crile concludes, "at the present time we are probably only on the boundary line of knowledge concerning the different constituents of the blood and their reactions, - so that we can not feel very sure of our ground until more research work has been undertaken and the results tested by time." He complains of the difficulty in securing reliable hemolysis tests and felt that he was left straddling between two theories of immunity: the physicochemical of Bordet and the purely chemical side chain hypothosis of Ehrlich. At that time no simple method for clinically determining the compatability or incompatability of blood for clinical use had been worked out. A rather elaborate setup for the determination of hemolyis, not agglutination, requiring about twenty-four hours was the technique used for testing the donors and Crile states that the occurence of hemolysis in vitro before transfusion does not even then necessarily indicate that it will occur in the vascular system of the recipient after the transfusion. Epstein and Ottenberg in 1908 were the first to apply agglutination tests in the giving of transfusions and to develop clinical methods for typing human bloods. Ottenberg (306) was likewise the first to suggest that the blood groups might be inherited.

Not until 1910 did the principles of isoagglutination begin to find real use in clinical medicine. Two publications

were responsible for its more widespread application. Landsteiner gave a complete description of the four groups in a monograph which appeared as a section of Oppenheimer's "Handbuch der Biochemie" (1910) and Moss (1910) presented the results of his most careful work and independently offered a classification of the blood groups which included four divisions depending on the reactions of the cells and sera with one another.

Unfortunately, he did not know of Jansky's work at this time and in assigning numbers to his groups he placed in his group I, bloods which Jansky had placed in his group IV, and vice-versa, a mischance which has and still does cause much confusion at times. Where Landsteiner had postulated only two agglutinins to account for the reactions he had observed, Moss felt that there were at least three isoagglutinins and three isohemolysins to account for the reactions as described by Landsteiner and the exceptions which he had noted in a large series of tests carried out on two hundred thirteen individuals, some healthy and some diseased.

To von Dungern and Hirschfeld (1910) goes the credit for introducing the nomenclature which at present time has the most widespread acceptance, the International Classification. They showed that these agglutinable properties or substances were inherited as simple mendelian dominants and called the

substance in Landsteiner's group A, agglutinogen A; and that in group B, agglutinogen B; and because group C apparently had neither A nor B, group zero or O. It naturally followed that if the group had both A and B it was known as AB. A comparison of the erminology and the contents at the end of 1910, which remains essentially the same today, is as follows:

Jansky	Moss	von Dungern and Hirschfeld	Agglutinogen	Agglutinins
I	IV	O	-	a,b
II	II	A	A	b
III	III	B	B	a
IV	I	AB	A,B	-

(8) The Heredity of Blood Groups

After a most careful study of seventy-two families and three hundred forty-eight persons, von Dungern and Hirschfeld (108) enunciated what has since become known as the first law of heredity; i.e., agglutinogens A and B can not appear in the children if they did not exist in the parents. They felt that the heredity of the agglutinogens depended on two independent pairs of allelomorphic genes A and a, and B and b, with A and B the dominant factors. Hence, the genotype of a person of phenotype A would be AAbb; of B, aaBB; of AB, AABB; while group O as a homogeneous genotype with all

the recessives would show aabb.

This theory was uncontested until Bernstein (404), in 1924, pointed out that when the theory of von Dungern and Hirschfeld is analyzed from the statistical standpoint the blood groups do not conform to expectancy. He suggested that instead of two pairs of allelomorphic genes, A and B, there were really three allelomorphic genes, A, B, and R. For many years a great number of exceptions were found to this law; but, with the gradually improving technique of grouping and cross-matching, the exceptions have become fewer and fewer so that it now seems generally accepted as a part of the law of heredity, when applied to blood groups, that a mother and father both of group O can not have a child of group AB, nor can the father and mother of group AB have a child of group O. Wiener (1939) has shown that the exceptions to the rule are, in statistics covering 4,568 mothers and 5,908 children, as follows:

Group O	children from AB mothers	0.33 per cent
Group AB	children from O mothers	0.1 per cent

Thomsen (1929) has demonstrated that the sensitivity of agglutinogen A is less than that of agglutinogen B in the group AB so that it is possible that in some of the listed exceptions to the rule proper grouping was not accomplished because it was based on the examination of blood cells alone which may give erroneous results because of the

diminished sensitivity of agglutinogen A. A further cause of discrepancies in the earlier reports undoubtedly lies in the failure of the investigators to realize the significance of the agglutinin titer in various serums, or realizing that there was significance failed to recheck apparent exceptions with sera of suitable titer.

(3) The Subgroups of Agglutinogen A

Von Dungern and Hirschfeld not only pointed out the hereditary character of the blood groups, offered a theory for their appearance in the population, and called attention to the medico-legal import of their findings, but also a little later (1911) presented the first evidence that perhaps group A was made up of two groups or subgroups which they called A_1 and A_2. These subgroups were substantiated and more carefully studies by Schütze in 1921, Coca and Klein in 1923, and later in the same year by Guthrie and Huck. The last named authors in an excellent piece of work concluded that the view concerning the existance of only four groups was incorrect; that only two isoagglutinins and two isoagglutinogens were insufficient to account for the findings; that there were at least three of each, maybe more, and that twenty-seven combinations are possible.

The first studies on the heredity of the subgroups

were made by Landsteiner and Levine in 1927 who believed that the differences were not only quantitative but qualitative in contradistinction to Lattes (250) who felt that the intragroup differences were only quantitative in character. Thomsen, Friedenreich, and Worsaae (1930) extended Bernstein's theory, contending that instead of three allelomorphic genes A, B, and R, there are really four allelomorphic genes A_1, A_2, B, and R with A_1 dominant over A_2; and A_1, A_2, and B dominant over R; but they did not challenge the original concept.

(4) The Racial Distribution of Blood Groups

Out of the continued interest in the serology of the blood groups a whole new field of anthropological investigation came into being with the announcement of Ludwik and Hanka Hirschfeld in 1919 that there were marked differences in the incidence of the various blood groups in the various races of men. These investigators, working in the medical corps of the Royal Serbian Army on the Macedonian front during the latter stages of the first world war, had opportunity to examine the soldiers of many races and the differences they found in the inherited biochemical group characteristics were reported as follows:

	A	B	AB	O
English	43.4	7.2	3.0	46.4
French	42.6	11.2	3.0	43.2
Italians	38.0	11.0	3.8	47.2
Germans	43.0	12.0	5.0	40.0
Austrians	40.0	10.0	8.0	42.0
Serbians	41.8	15.6	4.6	38.0
Greeks	41.6	16.2	4.0	38.2
Bulgarians	40.6	14.2	6.2	39.0
Arabs	32.4	19.0	5.0	43.6
Turks	38.0	18.6	6.6	36.8
Russians	31.2	21.8	6.3	40.7
Jews	33.0	23.2	5.0	38.8
Madagascians	26.2	23.7	4.5	45.5
Negroes (Senegal)	22.6	29.2	5.0	43.2
Annamese	22.4	28.4	7.2	42.0
Indians	19.0	41.2	8.5	31.3

They established what is known as the biochemical racial index; i. e., the ratio between A and B groups, and found that the ratio ap eared to be highest in the northern European people, lowest in the Asiatic and African races with the Mediterranean races intermediate in position.

Innumerable studies have been done since that time; nearly all of the races of the world have been grouped and many theories have been propounded to explain the findings and account for the distribution of human blood groups (409).

A survey of the literature indicates that men of all races have agglutinogens common to mankind in general. Given a blood sample, there is at present no agglutination test which will alone elucidate the race of the donor. Given large enough groups, however, certain striking differences

in frequency distribution will show up. The following list illustrates some of the extreme variations found in various parts of the world.

Distribution of Blood Groups
(Modified from Weiner, 1939)

Race	Blood Groups in Percentage			
	O	A	B	AB
Indians (Peru)	100.0	0.0	0.0	0.0
Indians (pure)	91.3	7.7	1.3	0.0
Negroes (Dutch Guinea)	83.0	0.0	17.0	0.0
Eskimos (Cape York)	80.7	12.9	2.4	4.0
Filipinos	64.7	14.7	19.6	1.0
Indians (Blackfeet and Blood Tribes)	22.3	76.7	0.0	1.0
Lapps (Sweden)	28.9	62.6	4.4	3.9
Hawaiians (pure)	36.5	60.8	2.2	0.5
Eskimos (East Greenland)	23.9	56.2	11.2	8.7
Australians (Southern)	43.8	56.2	0.0	0.0
Indians (Caroja)	39.0	5.0	51.0	5.0
Filipinos (Samel Moros)	25.9	18.1	44.9	11.1
Hindus (North)	31.3	19.0	41.2	8.5
Kalmuks	25.7	22.9	40.6	10.8
Gypsies (Hungary)	34.2	21.1	38.9	5.8

On thing seems obvious in a scheme like this: the more isolated a group of people, the more likely are their blood groups to swing to one extreme or another and remain there. Striking also is the fact that one set of Indians were found to have only group O blood, one set the highest percentage of A, and a third the highest of B.

(5) Agglutinins M and N

The iso-antibodies described up to this point which are useful in differentiating antigenic difference between tissue components of individuals belonging to the same species occur naturally and in man are a part of his inherited physiological

patterns. Erhlich and Morgenroth, however, at an early stage (1900) had shown that such antibodies might also arise as the result of artificial immunization. Extending this method of experimentation, Landsteiner and Levine in 1927 obtained immune serum from rabbits by injecting them with human blood and then when, by adsorbing or removing the agglutinins as they had previously done in differentiating the subgroups in human sera, all of the known agglutinins had been removed there still remained substances which could cause agglutination of most of the bloods of all four groups. Fitting theory to the findings, the investigators postulated two new agglutinogens and named the M and N. They apparently bear no relation to the agglutinogens A and B; and since no agglutinins capable of causing their coagulation have been found in normal sera, they are of no clinical significance in so far as incompatibilities in the practice of transfuions are concerned. They do, however, add another instrument of great precision to the armamentarium of the geneticist and medico-legal expert, for each of the four major groups may now be divided and followed in terms of its M and N content. All bloods have one or both of these antigens, which are inherited as simple mendelian dominants and their heredity, according to Landsteiner and Levine (1928), probably depends on a single pair of allelomorphic genes. There can be, there-

fore, according to this theory only three genotypes MM, Mn, and NN.

Wiener (1939) states that of the 10,438 mother-child combinations reviewed for his monograph not once have the combinations type M mother with type N child or type N mother with type M child been encountered. In each case where an exception has been made the parent involved was the father, so that illegitimacy seems a legitimate question to raise in accounting for these exceptions.

With this evidence Hirschfeld (1938) seems justified in adding to his first two laws of inheritance the following:

3. Agglutinogen M can not appear in the blood of a child unless present in the blood of one or both parents; and, likewise, agglutinogen N can not appear in the child unless present in the parents.

4. A type M parent can not give rise to a type N child; and conversely a type N parent can not give rise to a type M child.

One can readily envision where this sort of thing is leading. At present agglutinable substances in human blood cells may be demonstrated by normal human serum,- for practical purposes of blood transfusion this is sufficient,- by normal animal sera, by sera of animals immunized with human blood and by sera of human beings immunized with the blood of other individuals.

An agglutinogen or group of agglutinogens called P

has been described by Landsteiner and Levine (247), an agglutinogen q by Furuhata (404), an agglutinogen H by Schiff (350), and further individual differences continue to show up. It will take years to exhaust the animal and bird kingdoms of possibilities, and each new finding will make more complex the theory behind safe transfusions. Most of these findings will not play an immediate role in clinical medicine or directly reduce the hazards of transfusions, but they are important especially in understanding the phenomenon of hemolytic crises and even death of patients who according to all obtainable data have had bloods given them which should have been entirely innocuous.

(6) Intragroup Incompatibility

Wiener and Peters (405), in an unpublished paper, concern themselves with this problem of intragroup incompatibility.

Investigating the death of a patient, group O, after five transfusions with apparently compatible group O bloods, it was demonstrated that on direct cross-matching of the donor's serum with the patient's cells no agglutination took place, but when the donor's cells were place in the recipient's serum, by a special technique, agglutination could be demonstrated. Auto-agglutination was ruled out; therefore, the only conclusion justifiable was that the patient's serum

contained some special agglutinin entirely unrelated to previously known agglutinins in human sera and on repeated tests on O bloods, most of which were clumped, the agglutinogen did not seem to be A, B, P, M, or N.

The reactions did coincide remarkably with those given by certain antirhesus immune rabbit sera described by Landsteiner and Wiener to define an agglutinable property of human blood which they have seen fit to call Rh (248). The authors attribute the catastrophe in this case to the gradual build-up of anti-Rh antibodies in the serum of the patient to a higher and higher titer until after the fifth transfusion of Rh+ blood an acute hemolytic crisis took place.

A second case presented more evidence of the possible presence of weak anti-Rh in certain individuals, undetectable by any ordinary methods of cross-matching, yet capable of being enhanced in titer by transfusions in Rh+ bloods, especially repeated transfusions from the same donor. It, too, gave serological reactions similar in every respect to those of the antirhesus immune rabbit sera.

Just why this type of reaction does not show up more often, or in truth why it should appear at all, is a question the future must answer. This does seem certain,- that hemolytic reactions can and do occur after transfusions of the proper group due to agglutinogens unrelated to the four blood groups. This is most likely to occur where repeated

transfusions are given and there is built up in the patient's plasma iso-antibodies for the donor's cells so that on the re-introduction of those cells at some later date there is an acute reaction very similar to the anaphylactic shock sometimes seen upon the delayed introduction of other foreign protein into an individual.

The greatest number of intragroup hemolytic reactions seem to occur in women who have recently become mothers or have had a miscarriage.

Levine and Stetson (256) have suggested that the fetus inherits an antigenic substance from the father which is lacking in the mother and that the mother becomes immunized against this substance after carrying the child for a long time, especially in the presence of pathology which would break down the normal barrier in the placenta between the mother and the child, so that when the mother receives blood cells in a transfusion for which she carries an antibody in her serum, the inevitable result is a reaction.

Wiener postulates that if the presence of agglutinogen A or B in an O mother can engender in her serum high titered iso-antibodies for A or B, then it does not seem improbable that an Rh- mother carrying a Rh+ child might react by producing Rh antibodies. Many hundreds of thousands of successful transfusions suggest that this must be a rare occurrence.

This new agglutinogen Rh is different from the agglutinogens A_1, A_2, P, M, and N if these early observations are confirmed, for none of those mentioned above has been proven to be the cause of a fatal hemolytic reaction in man. While these first two cases on record concerning the formation of specific antibodies by the substance Rh ended in the death of the patients.

(7) Complexity of the Problem of Groups.

At present with the agglutinogens A_1, A_2, B, M, N, P, and Rh alone, seventy-two different types of human blood are readily distinguishable . There is little hope that the passage of time will make the problem less complex unless the nature of the substances is defined and some common denominator of a chemical type can be shown to underlie the varied biological manifestations of specificity.

At the time of this writing, bloods which have been preserved too long in this laboratory for safe transfusions are being turned over to Doctor Landsteiner for use in the continuous search for the nature of these substances which hold in part the answer to questions of the nature of life itself.

CHAPTER II

Part IV

Development of Technique

(1) Cannulas

Libavius in 1615 gave an accurate description of the technique of blood transfusion as follows: "Let there be a young man, robust, full of spiritus blood, and also a man, thin, emaciated, his strength exhausted, hardly able to retain his own soul. Let the performer of the operation have two silver tubes fitting into each other. Let him open the artery of the young man and put into it one of the tubes fastening it in. Let him immediately open the artery of the old man, and put the female tube into it, and then the two tubes being joined together, the hot and spiritus blood of the young man will pour into the old one as if from a fountain of life and all of his weakness will be dispelled" (414).

Bernheim in 1917 wrote; "the instrument which I shall present is one of my own design. --It is a two pieced affair consisting of two hollow tubes, each 4 cm. long, and each bulbous at one end in order to form a neck for retaining the tie or specially devised clamp; the other ends are tubular and fitted for invagination.-- The radial artery of the donor is usually united to one of the superficial veins at the

elbow of the recipient. Where the recipient is practically exsanguinated, it is wise to give him all the blood he can conveniently hold."

Three hundred years separate these two descriptions; yet ther is little to choose between them except Bernheim's suggestion that the recipient's vein be used rather then the artery.

There have been literally hundreds of types of apparatus for both direct and indirect transfusions. In France, Robert des Gabets (1653) had a fellow monk make an apparatus consisting of two little silver tubes which were connected by a leather ball the size of a walnut. Each tube contained a valve to regulate the flow of blood. Pressure on the ball supplied the force necessary for continuous flow in one direction from vein to vein (340).

In 1654 Folli of Florence proposed a silver tube to be inserted into the artery of the donor, a cannula of bone to be inserted into the vein of the recipient, and a connecting piece, made from the blood vessel of an animal, which had a side arm to allow the escape of air. Technically, everything necessary for a successful transfusion was thoroughly understood, it seems, even at this early date. The knowledge of bacteria and isoagglutinogens was lacking, and in this deficiency lies the difference in results (281).

Even in 1664 Major (62) realized that corruption was less frequent if the skin over the vessels was thoroughly cleansed with alcohol in the form of spirits or wine before infusorial surgery was practiced.

Among the more interesting instruments historically considered are: descendants of Libavius' apparatus in the form of the segmented metal cannules of Lower (1666); the Gesellius (1873) cannula which had stoppers attached to each segment to make the operation less bloody and prevent undue hemorrhage; and the later refinements of Bernheim (30).

(2) The Syringe Principle

Blundell (1818) introduced the valve syringe principle and used it with his Impellor, a rather large funnel-like container which could be attached to the back of the chair in which the recipient sat. As the blood flowed into the receptacle it could be forced on into the recipient's arm by a pump motion and a series of valves made of leather and steel springs. This pump and funnel arrangement showed up in 1862 in the Hematophore of Moncoq, the syringe and funnel of Gross (1866), the Collin apparatus (1874), and Oré's modification (1875) (For illustrations see Zimmerman and Howell, 414)

(3) Capillary Blood Apparatus

A new departure was seen in the Capillary Blood Transfusion Apparatus of Gesellius (1873) which consisted of a large, diving helmet-like jar which could be held to the patient's back while a multiple lanceted plunger was sharply pushed against the skin to cause miltiple bleeding points. When sufficient blood had run into the jar it was forced out by a syringe mechanism through a pipe into the vein of the recipient. Von Ziemssen (1892) introduced the multiple syringe method which Lindeman (263) popularized through the introduction of his cannulas with sharp styletes. The latter could easily pierce the skin without any surgical procedure, guide the blunt beveled cannula into the veins, and then be removed. This method became widespread in use because it allowed unmodified blood to be used without sacrificing the artery of the donor and the vein of the recipient.

The Aveling apparatus (1863) is an almost perfect reproduction of that used by the old French monk one hundred years before. McGrath enlarged the size of the pressure bulb and re-introduced the principle in 1914 (414).

(4) Principle of Hydrostatic Pressure

In 1877 Esmarch (114) introduced a simple, elongated,

glass flask with a funnel shaped end for use in giving defibrinated blood by hydrostatic pressure. This is the prototype of nearly all modern intravenous infusion sets. Its simplicity made it the favorite type of apparatus for all of the early users of blood stabilized by anticoagulants according to the teachings of Lewisohn (259). Later, modified by Robertson (1918), it prevented undue exposure of the blood by creating a closed system. In various forms, it is the basic type of apparatus in use at present.

(3) Blood Vessel Anastomosis

Such ease of use, however, was not a sudden jump from Ksmarch to the present. The problem of blood transfusions has intrigued the surgical and mechanical ingenuity of numbers of men. With the growth of aseptic surgery, Carrel (71) in 1905 perfected a method of anastomosing the artery of the donor to the vein of a recipient, eliminating contact with all foreign material and presenting an unbroken endothelium over which the blood could pass, thereby greatly reducing the chances of clotting. This was a delicate and difficult operation; not always successful in the hands of even skillful surgeons, out of the question for general use.

Crile, 1909, made this operation easier by using a small cannula through which the vein of the recipient was drawn and cuffed back, allowing the artery to be telescoped

over the outturned endothelium of the vein to form a continuous lining of intima.

Elsberg (1909), Soresi (1911), and Jeger (1913) modified this method but the method fell into disuse because of the delicacy and difficulty of the operation, the danger to the donor, and the inability to estimate the amount of blood transfused (414).

(6) Paraffined Containers

Other men attempted to get better results by paraffining the containers. Chief among these were David and Curtis (1912), Kimpton and Brown (1913), Satterlee and Hooker (1914), Percy (1915), and Kreuscher (1918).

(7) Athrombit

Neubauer and Lempert in 1930 introduced a container made of an amber-like condensation product of phenol and formaldehyde called "athrombit." Bürkle-de la Camp (67) constructed a flask of this material almost exactly like Percy's modification of the Kimpton-Brown tube and it is this tube which is used without anticoagulant in Germany on a large scale.

(8) Switches and Valves

Unger (1915) introduced a syringe cannula apparatus with

a four-way stopcock arrangement which allowed outlets to the donor, the recipient, and a bowl of saline for flushing to prevent clots. Innumerable variations on this theme of complex switches and valves have been introduced, among them Brines (60), Feinblatt (122), Scannell (347), Soresi (374, Koster (231), Cashman and Baker (72), and Pennell (313). Beck (24), who invented one of the more elaborate machines, has many interesting reporductions of the older ones. Most of them have failed by virtue of their elaborateness and complexity, but still they come.

We go back again for guidance to Hueter who in 1870 said: "Too much stress is laid on the technic as in all operations which are still in the stage of infancy. Of all the instruments only the simplest are kept."

CHAPTER II

Part V

Anticoagulants

(1) Early Use

The prolonged preservation of donor blood depends largely on the quality of the anticoagulant.

The use of anticoagulants in blood seems to have had its inception in that stormy four years, 1664-1668, which really mark the beginning of modern experimental medicine. J. D. Major (62), who contested Lower's claim to fame as the first animal transfusionist, used ammonium sulfate to prevent coagulation of the blood in 1667. Transfusions soon fell into ill repute and so the method did not grow.

Soon after Blundell (1824) revived interest in the operation, defibrinated blood came into wider usage for transfusions and was the method of choice until Köhler in 1877 pointed out the possible dangers of intravascular clotting following its administration (102).

Braxton Hicks (1868) successfully used sodium phosphate in his obstetrical practice but gave up the method because of the apparent toxicity of blood so stabilized.

At about the same time or a little later, 1875-1880, physiological salt solution, introduced by Latta in 1831, practically replaced blood in the treatment of acute anemia,

hemorrhage, and shock.

Landois introduced hirudin as an anticoagulant in 1892, and it proved very effective for investigation in vitro of blood for it neither changes the cell volume nor the specific gravity of the plasma, but its source was so limited and its toxicity so variable that its use for humans has not grown (1, 345).

In an attempt to get more uniform results with the capillary hematocrit of Blix, introduced by Hedin in 1891, many attempts to find suitable anticoagulants were tried. Daland (1891) tried 2.5 per cent potassium bichromate, Biernacki (1894) used a mixture of oxalic acid and sodium oxalate, while Koeppe (1895) found that blood could be prevented from clotting for a considerable period if the tube contained cedar oil, and Herz in 1893 used codliver oil to effect the same end.

(g) Priority in the Use of Sodium Citrate

Sabattani, in 1900, suggested that sodium citrate had an advantage over oxalates and fluorides as an anticoagulant because it simply immobilized the calcium necessary for clotting instead of creating an insoluable precipitate. It reacts in the ratio of three molecules of sodium citrate to one atom of calcium. (132).

Safe indirect transfusions date from the introduction of sodium citrate on a large scale into the practice of blood transfusions. The method was thrown into the medical spotlight by the war (1914) with its attendant demand for effective methods of treating the wounded. Some controversy arose about the honor of priority as regards the introduction of this method.

Search of the original papers establishes without much doubt that Andre Hustin of Brussels not only suggested the use of citrated blood for transfusion in April of 1914, but in May of the same year reported to the Belgian Surgical Society experiments testing the rate of coagulation of blood in isotonic saline, in Ringer's solution, in five per cent glucose solution, in citrate and physiological saline, and in a solution of 5 per cent glucose in saline to which sodium citrate had been added to make a 2.5 per cent solution. The last he pronounced as best and then reported results on four types of transfusions using this preservative.

1. From one dog to another.
2. From one Rabbit to another.
3. From man to dog.
4. From man to man.

He listed the advantages of the method as being; no surgical operation, known amount of blood given, less danger for donor, many donors for same patient, blood could be oxygenated or treated with other therapeutic agents, glucose good for cells,

infusion can be given slowly, and coagulation time of recipient's blood actually shortened (189).

On December 17, 1914, Richard Weil of the General Memorial Hospital of New York reported at the Academy of Medicine the results of forty-three transfusions given by the indirect method with the aid of 10.0 cc. of a 10 per cent solution of sodium citrate in saline for each 100.0 cc. of blood. This work appeared in print on January 30, 1915 (403).

For us, in considering the origins of the idea of preserved blood for transfusion, Weil's work is of perhaps the greatest importance because he not only started the work in America but was the very first to use blood kept for three to five days, first to cross-group blood ahead of time and keep it on hand where the possibility of hemorrhage existed, and first to report the morphology of cells kept for a week or more to determine the rapidity of change under such conditions of preservation.

At the same time that Hustin was carrying out his work in Belgium and Weil in America, Agote was carrying on independently in Buenos Aires and presented his work in November, 1914. In January, 1915, he published his results under the title of "A New Procedure for the Transfusion of Blood."

Richard Lewisohn published in New York the first of a

long list of papers on the citrate method on January 23, 1915 (259). He knew of Hustin's work and had heard Weil speak. He has, therefore, no claim to priority but to him more than to any other individual goes the credit for popularizing blood transfusions by the so-called "medical procedure," for simplifying the technique, and putting the practice on a thoroughly workable basis. He has been the greatest protagonist for and defender of the citrate method.

Fischer introduced the method in German literature (128). In March, 1916, he reported comparative studies on hirudin, sodium citrate, sodium oxalate, peptone and glucose mixtures with the direct methods in relation to use in the German armies. On a whole, they adhered to direct transfusion.

Hédon (1902), under the impression that the toxic substance in blood was carried in the serum, had been the first investigator to remove and then resuspend the cells in physiological saline for transfusions. He, likewise, was one of the first to point out the advantage of using defibrinated blood several hours old. After man independent studies, in 1917 he introduced to French literature the citrate method of blood conservation; and Jeanbreau, at that time Chief of the French Army Ambulance Service, put it into immediate practical use at the army bases. In July, 1917, he reported the results in his first eleven cases (192).

During the same month, Stansfeld (375) advocated the method for the English Army, but the British Medical Committee was so interested in the use of gum acacia recommended by Bayliss (22) as a substitute for blood in the treatment of shock and hemorrhage that this new and simpler method of transfusions was overlooked for a long time.

Since Hustin's first attempt to find the most suitable method of using citrate, there have been many modifications of the solutions in which it was basic. There have been attempts to buffer the solution by the addition of slightly acid salts as Grey (1937) did. The best known of the physiological citrate solutions is the I. H. T. solution Belachovsky prepared at the Institute for Hematology and Transfusion of Moscow, sometimes referred to as the Central Hematology Institute. It contained sodium citrate, sodium chloride, potassium chloride, and magnesium sulfate in an isotonic solution.

With the advent of the American forces into the war, Robertson (337) introduced at first the procedure elaborated by Rous and Turner in 1916 which consisted of preserving the cells for later transfusions by adding 100.0 cc. of 3.8 per cent sodium citrate and 150.0 cc. of 5.0 per cent glucose to each 100.0 cc. of blood. Hemolysis was minimal in this preservative, but the amount of citrate present in a trans-

fusion of any size precluded its use for immediate transfusion into human beings. The cells were resuspended in saline just before use and a small series of transfusions was carried out with these preserved cells. This procedure did not work out well in actual practice and Robertson changed to the citrate method already in use by the French. He supplied front line hospitals of the American Expeditionary Forces with compact sets consisting of a stoppered bottle containing sterile citrate solutions and an Ingram syringe for creating either pressure or suction so that the blood could be received into and dispensed from the same container without handling. On April 29, 1918, his first report of its use in forty-four cases appeared (336).

(3) Citrated Blood versus Unmodified Blood

With the introduction of this new method of transfusion, there arose the question of the relative merits of modified blood and unmodified blood. Lewisohn (1916) reported excellent results with the method. His reactions (19 per cent) were less than those reported by Lindeman, in 1914, who used the multiple syringe direct method. Garbat felt that 0.25 per cent was surer than 0.2 per cent and declared the method safe.

Syderstricker, Mason and Rivers (1917) confirmed

Lewisohn's work and reported 17 per cent reactions with citrated bloods of all types.

All of the reports, however, were not so favorable, for in the same year Meleney, Stearns, Fortuni, and Ferry (285), in a report of two hundred eighty transfusions, placed their reactions following transfusions with multiple syringe method at 64.4 per cent, with almost identical figures for the citrate method, 64.0 per cent. They concluded that the method played no part in the occurence of the reactions; that neither did any good in the case of severe infection or bacteremia; that small transfusions had less tendency to be followed by reactions and the highest incidence was found in cases of repeated transfusions, especially if the donors were used repeatedly.

Drinker and Brittingham (1919) reported 60 per cent reaction and stated: "We should count ourselves fortunate could we reduce reactions from transfusions of whole citrated blood to 45 per cent." Unger (1921) who became one of the method's early antagonists likewise reported an incidence of 60 per cent. Bernheim (1921), Brines (1923), Bacon (1924) added their warning that the method was dangerous, while Hoffman (1922), Joannides and Cameron (1924) felt that the danger was over stressed. Eichner (1927) felt that the limits of safety were even greater than some of the earlier

authors had indicated.

In this country reactions since 1930 have dropped so precipitously from the average high incidence before that time that some factor besides the citrate itself must have played a role in this sudden improvement. All of the older writers had expected chills following transfusions, and when intravenous saline displaced transfusions to a degree no alarm was felt when they too were followed by reactions of varying degree. This attitude is epitomized by Crile who says in his book published in 1909 when speaking of the course following a transfusion; "A chill of greater or less severity followed by a corresponding febrile reaction is to be expected, and usually occurs. Ordinarily, it apparently has no more significance than the chill which frequently follows the infusion of saline solution."

Not until salvarsan was introduced into medicine and intravenous injections became widespreed was attention sharply focused on the "water fever" as Wechselmann called it when he first demonstrated in 1911 that the water itself was the cause of the reaction and not the drug. He demonstrated many actual bacteria and other growths of a fungus-like nature in the distilled water used (402).

Hort and Penfold, the same year, demonstrated that

"pyrogenic" substances were often present even when no bacteria were demonstrable. It was shown that they developed on standing; were filterable; and gave two types of fever, one immediate and transitive, the other delayed but continuous; probably were of bacterial origin but were not actually bacteria (185).

This most important piece of work was overlooked until Seibert in 1923 demonstrated that these "pyrogens" were actually the protein end products of certain river water bacteria which were more prevalent in certain seasons than in others. She carefully ruled out bacteria and specific iron effect as the cause and went on to show that a still outfitted with a proper baffle could prevent the carrying over of these substances into the distillate (360). Fantus, reviewing this phase of the work in 1926, pointed out that not only must the water be freshly distilled but sufficient metal could be dissolved from lead, copper, nickel, or zinc stills to cause toxic manifestations. Not only were glass stills preferable; but, quoting Matzenaurer, he points out that enough alkali may be dissolved from ordinary glass to cause toxic reactions (119). This work brought into prominence the observations of Busman (68) that the sulfur in certain types of rubber tubing used for intravenous work likewise would cause reactions. These studies removed some of the odium

from the anticoagulant, but there remains the question of its action on the blood constituents and this becomes of increasing interest when the matter of prolonged preservation is considered.

All of this information was slowly put into use. It seems certain that it is as untenable to try to compare the incidence of nonfatal febrile reactions following citrated blood transfusions before 1925 and after 1930 as it is to compare justly the incidence of hemolytic crises before and after the discovery of the blood groups.

(4) The Action of Sodium Citrate

No better explanation of the specific toxic action of sodium citrate upon introduction into the blood stream has been offered than that of Vietinghoff-Scheel. In 1902, he postulated that the toxicity was due to immobilization of the calcium ion in the tissue, in a manner similar to that in which Sabattani had shown it is immobilized in the serum, thereby producing a clinical effect similar to that produced by a true hypocalcemia. This was especially true as regards the reaction of the nerves and muscles in the presence of an excess of citrate (397).

Gros in 1913 observed a slowing of a frog's heart on which sodium citrate had been poured. Salant and Hecht

in 1915 compared the influence of of citrates, as well as oxalates and tartrates, by perfusion experiments on the isolated hearts of other animals and observed following citrates slowing similar to that observed on the frog's heart.

Lewisoln, guided by Hustin's and Weil's work, established 0.2 per cent as the smallest dose that would keep blood from clotting for thirty minutes. He established 5 grams for an individual transfusion as the upper limit of wise therapeutic use, 10 grams as definitely in the toxic realm, and 15 to 20 grams as probably a fatal dose (260).

Garbat (1916) repeated Lewisohn's experiment and concluded that a 0.25 per cent citrate solution is more likely to prevent coagulation, without increasing the danger. He set the fatal dose for humans as between 15 to 20 grams (135).

Salant and Wise (1916),in a cereful investigation of citrate and its decomposition in the body, put the fatal dose between 0.4 and 1.5 grams per kilogram; the results varying greatly in the different experimental animals used and appeared particularly dependent on the rate of injection. They concluded that citrate disappears rapidly from circulation and is most toxic in those animals in which large quantities were eliminated unchanged. Overdosage caused only acute poisoning varying in intensity with the rate of oxidation. No subacute or chronic effects could be demonstrated (342).

Drinker and Brittingham (105) after testing many types of citrate solutions discarded the idea that the toxic factor in the blood was due to "fibrin ferments" in the plasma or "potential coagulative" factors so advocated by Satterlee and Hooker (345) for they found "citrated plasma, thoroughly freed from all formed elements, is singularly nontoxic." They felt that aside from the direct toxic action of citrate, the platelets freed a toxic substance when they were broken down.

Unger (1921) ascribed the unfavorable reaction not to changes in platelets alone but to changes in all of the cellular elements of the blood. From the erythrocytes, he felt, it extracted a substance which is able to inactivate complement by the creation of an anticomplementary substance. He reported that leukocytes was destroyed, the red cells were made more fragile, the opsonins were reduced to zero and complement was diminished by direct action. Many of these findings have been substantiated by subsequent observers (chapter III) but their part in the causation of reactions is not a proven one.

There were many who felt that citrated bloods were dangerous and caused an unnecessarily high percentage of reactions (Bernheim, 1921; Brines, 1923; Lederer, 1923; Bacon, 1924; Kretzler, 1924; Pauchet and Becart, 1924), and while many transfusions were reported in the late 1920's few attempts were made to elucidate further the mechanism or offer

suggestions for preventing the changes supposedly associated with increasing toxicity.

Joannides and Cameron (1924), in an experimental study on dogs, observed that overdosage was followed by convulsions, respiratory slowing, and cardiac failure; while Shelling and Maslow (1928) observed death in their rabbits following the feeding of citrate in milk by mouth.

Minot, Dodd, and Bryan in 1933, having observed the death of a marantic child following a citrate transfusion concluded that since in infantile tetany calcium was reduced, the citrate probably further reduced it by forming an unionized complex in the plasma, and by increasing the alkalinity of the blood in two ways: by direct addition of the very alkaline solution and by increased bicarbonate formation as a result of the oxidation of the preservative. They produced experimentally relative degrees of hypocalcemia by parathyroidectomizing dogs, injecting quanidine, and by injecting soda bicarbonate. In each instance the addition of citrated blood or isotonic sodium citrate equivalent to the amount usually given in a transfusion (10 cc. of 0.5 per cent citrate blood per pound of dog) caused slowing of pulse and respirations, twitching, vomiting, and death if the infusion was continued. In each case, if the dog at the level of unconsciousness was given 3 to 10 cc. of 10 per cent calcium gluconate, relief was almost immediate. These authors, therefore, recommend the giving of calcium

gluconate, prophylactically, when citrated bloods are to be used in states which are likely to be associated with a hypocalcemia (288).

As a good demonstration of the ever recurring cycle of things Shafer and Crismon in 1936, having observed the paralyzing effect of citrate on a frog's heart, perfused cats and demonstrated that first the vagus becomes paralyzed; the action taking place at the synapses between the pre and post gangliomic fibers, then the heart muscle becomes directly affected, venous pressure goes up, arterial pressure down, and the heart stops in diastole if calcium salts are not quickly added (362).

There is evidence, therefore, that citrate immobilizes calcium in the tissues and causes a picture comparable to acute calcium deficiency as Vietinghoff-Scheel had pointed out in 1902.

(5) Heparin

Heparin has been used as an anticoagulant in two ways: in vitro in a manner similar to sodium citrate (Skold, 1936; Tretow, 1937; Schürch, 1938; Clemens, 1938), and in vivo by rendering the cells of the blood of the donor incoagulable by intravenous injections of heparin a short time before drawing the blood (Hedenius, 1936, 1937; Knoll and Schürch, 1938; Seppington, 1939).

It is an active fraction of the naturally occurring anticoagulants, first isolated by McLean working in Howell's laboratory at Johns Hopkins Hospital in 1916 and named by the latter heparin because it was found in the liver.

The purification of the substance offered some difficulties, however, and Mason (1924) who tried it in transfusions gave it up because of the reactions. He reported that in doses of 100 milligrams it was toxic for man.

Howell obtained a purer product in 1928 and reported good results on ten patients. Best and McHenry began investigations with the hope of obtaining active fractions in the same year (32).

Charles and Scott (75) carried on this investigation at Toronto and in 1933 obtained a crystalline barium salt of uniform potency. The same year Schmitz and Fischer (352) reported from Copenhagen a purified product and discussed the chemical composition of the substance in some detail.

Jorpes in 1935 suggested that the substance was a mucoitin polysulfuric ester, and noted that the loss of the sulfuric acid groups seemed to interfere with its activity. Further investigation of its chemical properties have been carried out ~~in this clinic~~ by Chargaff and Olsen (1937) who showed that if heparin is given to an animal and is followed by protomine the effect of the heparin may be completely

neutralized and the clotting time brought back to normal rapidly.

Under physiological conditions it is apparently an antithrombase (Mellanby, 1934); under certain pathological conditions it may act as an antiprothrombinase (Howell, 1918); and it has been suggested by Waters, Markowitz, and Jaques (1938) that the increased clotting time seen in various types of shock may be due to increased liberation of heparin. This is extremely important from the point of view of using heparinized stored blood for transfusions in such cases.

The results, using heparin as a simple anticoagulent in vitro, do not seem striking in either one way or the other except that it requires much more heparin to keep the blood fluid enough to be easily transfused than it does just to prevent coagulation.

Sappington (1939) has pointed out that it requires 25 to 75 milligrams of heparin to stabilize 500 cc. of blood and that this quantity in every case exceeds the 0.25 milligrams per kilogram threshold-dose of the recipient, hence in every case the clotting time is increased from a normal of ten to twelve minutes to periods of from fifty minutes to two hours.

The second method of procedure consists of heparinizing the donor's blood by the injection of about 1.0 milligrams

per kilogram of body weight intravenously (1.5 cc. of the 5.0 per cent solution), waiting five to ten minutes, then withdrawing the blood and using it immediately for transfusing the patient. Hedenius has reported such success with the method that in Stockholm it has replaced the citrate method (168). This may be so, but at the present stage of development the use of heparin as an anticoagulant for routine transfusions is an expensive and potentially dangerous experiment; for certain special types of cases it offers undoubted advantages. The antidote for heparin is protamine, an amine derived from spermatozoa and fish spawn. Jorpes (1939) feels that it acts by removing the negative electric charge and causing flocculation.

(6) Other Preservatives

While citrate in one form or another has been the anticoagulant of choice in most instances, there have been trials with many other substances.

Roberti, Flandin, and Tzanck in 1921 introduced the use of sufarsenol and reported preservative properties greater than citrate with less toxicity (132).

Norton (1924) advocated sodium iodide while Brines (1926) advocated ammonium oxalate and arsphenamine, and in the same year MacCraken (60) used sodium sulfate successfully. One of the more interesting pieces of work in 1926 was that of

Perry who used lithium citrate with the definite idea of preserving the erythrocytes longer (316). She, like Rous and Turner, removed the cells from the preservative at the time of transfusion and resuspended them in normal saline.

Since 1935 there have been a number of articles from Italy advocating the use of sodium polyacetyl dioxysulfonate under the trade name of "Transfusol." Forti (1937) states that it acts as an antithrombin, does not alter the biochemistry, does not precipitate the calcium, does not lose it qualities on storing, is bacteriostatic, atoxic, and always ready for use in sterile vials of 5 cc. for each 100 cc. of blood. There have been reports by Lattes, Pieroni, Ceccia, and Morgaria sustaining these observations and if all of these reports are true, this is indeed an ideal anticoagulant (131).

CHAPTER II

Part VI

Cadaver Blood

(1) Origin of Its Use

Professor W. N. Shamov of Kharkov in 1927 became interested in the question of correcting defects in living bodies by the transplantation of tissue from the dead (364). He felt, as many before him had felt, that death is only the moment of dissolution of an intricate complex of mutual relations between the seperate tissues of an organism which individually retain their vitality for a varying time after the organism as a whole ceases to function.

In the first series of investigations, he and his assistant, Kostriukov, made hundreds of examinations on tissue and organs of animals from fifteen minutes to twelve days after death when the bodies were kept at 0°C. They observed that after ten to twelve days nearly all tissues became infected and quickly deteriorated. All infection seemed to sweep outward from the abdominal cavity, those tissues farthest removed from the intestinal tract remaining bacteria free longest. If there were other foci of infection at the time of death, they served as the starting points of waves of infection parallel to that from the intestine.

It was observed that when the tissues were kept close to

zero temperature even the peritoneum often remained free of infection for ten days while more distant organs, e.g., brain, bone marrow, and heart blood, were good after twelve or more days; while at 22° only the bones and marrow are sterile after twenty-four hours.

He concluded, therefore, that tissues from cadavers of previously healthy individuals may be used by surgeons, not only some hours but even days after death, if the bodies are kept at low temperatures.

Filatov (1937), taking this advice, has transplanted corneas from the dead to the eyes of the living with excellent results.

Tests to determine the amount of vitality and potential function in large tissue masses or organs were difficult to devise and carry out. The choice of a suitable tissue to work with was a problem; blood, as the easiest to handle, was chosen.

In 1929 a new series of experiments was set up using blood from chloroformed or strangled dogs (363). Contrary to expectancy, it was observed that such blood could be injected into other dogs with impunity. It had been thought that the blood would be flooded with bacteria and other toxic substances as a result of death and subsequent rapid autolysis of tissue.

In the first series of experiments when the dogs were killed by chloroform and kept at room temperature, it was

almost uniformly impossible to get sufficient blood to carry out experiments after the first four hours because of clotting. When, however, the animal was quickly strangled and immediately put in a refrigerator, free-flowing, unclotted blood could be removed many hours afterwards. Having shown that such blood was not toxic, the next step was to test the ability of such blood to carry on the functions of whole fresh blood.

To test the vitality of the blood, a third series of experiments were conducted in which blood from human cadavers was used to replenish the circulatory volume of dogs whose blood exhaustion had been raised 60 to 90 per cent by repeated saline infusions and bleedings. In every case there was recovery when saline alone no longer was effective.

These results left no doubt in the mind of the investigators that blood of a cadaver taken ten to twelve hours after death retains not only physical properties capable of sustaining circulatory balance but also, in so far as the erythrocytes are concerned, full functional ability.

(2) Clinical Application.

Professor S. S. Yudin of the Sklifosovsky Institute and Post Graduate Medical School of Moscow, knowing of Shamov's work, waited for sometime before a suitable opportunity

presented itself to try it out on a human being.

On March 23, 1930, with considerable trepidation he transfused an engineer, who had attempted suicide, with cadaver blood three days old which happened to be at hand and the man recovered promptly from the effects of his hemorrhage. Six more successful trials had been made when he presented the results before the Fourth Congress of Ukrainian Surgeons of Kharkov on September 7, 1930. He felt that with the approbation of this group he might proceed with an operation which was highly distasteful in many respects to the population as a whole. In November, 1932, he presented the results of his first one hundred cases (410).

There were many comments concerning this new departure in blood transfusions following this report (77, 228, 390).

(3) Vitality of Erythrocytes

Skudina and Barenboim (1931) working under Yudin repeated Shamov's earlier work and extended it to show experimentally the erythrocytes of fresh cadaver blood completely retained their role as oxygen carriers as measured by Barcroft's gas exchange method (369).

(4) Spontaneous Fibrinolysis

It was Skudina, in 1934, who noticed that the blood

collected from cadavers for Wassermann tests remained liquid even after many hours. The clot formed soon after removal from the body but within from fifteen minutes to one and a half hours later it seemed to dissolve. This was due, it was thought, to an actual lysis of the fibrin in the clot. Investigating the problem further with Rusakov (1934), the pathologist of the Institute, it was observed that this fibrinolysis took place only in the bloods of relatively healthy individuals who died suddenly; e.g., following accident or emergency operation. These changes did not take place in the bloods of those dying from chronic illnesses with cachexia and wasting, such as cancer, tuberculosis, or widespread sepsis of other types,(370).

At the time this observation was made Yudin had given about two hundred transfusions using 4 per cent sodium citrate as an anticoagulant.

The next eight hundred cases were done using only bloods in which fibrinolysis took place spontaneously, therefore needed no anticoagulant. The reactions in the second group were only 5 per cent compared to 20 per cent for those in which citrate had been used. Three things probably played a role in this lowered figure; increased experience; elimination of possible citrate reactions when large transfusions were given; and probably most important, the spontaneous fibrinolysis ruled out the use of bloods from bodies of persons who had

died of severe or chronic infection and the results of long wasting diseases (411).

(5) Probable Relation of Heparin to Fibrinolysis

One of the more important investigations in this series was the demonstration of a greatly lengthened clotting time in the blood of persons in severe traumatic shock by Bacharov (411). It recalls the observation of Biedl and Kraus in 1909 that the blood of dogs in anaphylactic shock had clotting times increased by many minutes to many hours. Waters, Markowitz, and Jaques have suggested that the increased clotting time in anaphylactic shock is due to the rapid liberation of heparin (400).

Perhaps the substance in cadaver blood that prevents clotting is heparin. Since heparin is apparently thrown into circulation in increased amounts only when a person is in shock, only those persons dying acutely will have it in the blood stream; while those dying quietly from sepsis or cachexia produce no heparin in the last moments, therefore have no anticoagulant to prevent clotting.

(6) Functional Separation of Portal and Caval Blood

In the development of the technique of cadaver blood transfusions, it was observed that blood from the portal system would not spontaneously drain from a cannula in the

internal jugular vein, even with the body in deep Trendelenburg position (370). The blood apparently comes only from the caval system and that from the mesenteric veins can be retrieved only by washing them through with saline. This is of both theoretical and practical interest because the blood in the mesenteric veins becomes infected soon after death, while that in the caval system may be infection free days after death.

(7) Phagocytosis

In 1935 Karavanov, carrying on the work at Kharkov, established the fact that phagocytosis is well preserved in cadaver blood for about eleven hours after the death of an animal, then disappears rapidly.(363).

(8) Reactions

In 1936, eight years after his original work, Shamov first tried cadaver transfusions in his own clinic. In forty-two cases reported by Karavanov, Karavanov, and Perelstein (217) there were reactions in 14 per cent. This is a striking figure because the senior author of that paper reported one hundred two transfusions of preserved donor blood from the same clinic the year before (216) and there were 67 per cent reactions, 26 per cent of which were severe, though blood was preserved only five to seven days. It was felt that in addition

to lessened reaction and lack of need of anticoagulant, the response on the part of the hematopoietic system of the patient was actually greater.

By 1938 Yudin (412) had done 2,500 transfusions of cadaver blood with seven deaths and 5 per cent reactions. An average of 1,500 cc. of blood was obtained from each cadaver, up to 3,000 cc. when the portal system was washed through with saline. He recommends ten days limit of storage since icterus followed use of older bloods. The operation in other hands has not yielded such excellent results. Arutianian and Shevedsky (1934) reported observations on fifty-two transfusions. Blood was collected from forty-four cadavers; twenty-eight were used, fourteen were unsuitable. Thirty-two liters were obtained and in fifty-two transfusions there were elevations in hemoglobin of 2 to 14 per cent and in red blood cells elevations of 800,000 to 1,000,000. The time limit for storage was fifteen days, yet in forty-two accurately studied cases there were reactions of forty, or 95 per cent of the cases; fourteen were severe, sixteen moderate, ten slight, and only two showed no reactions.

(9) Changes in Glucose, Lactic Acid, and Phosphorus.

Balakhovsky and Ginzburg (1934) investigated cadaver blood on the third, fourth, and sixth days from the beginning

of conservation. In contradistinction to donor blood, in cadaver blood there is a hyperglycemia; probably due to the action of liver on glycogen after death, for hepatectomized dogs failed to show this increased glucose level (17)

There was an increase in lactic acid at times which was greater than that which can be accounted for by glycolysis alone and the authors suggest that some other process causes breakdown of gluco-protein complexes. The lactic acid content had a range of 40 to 60 mg. per cent on the sixth day post-mortem. This range was found in donor blood after twenty-four to thirty days of storage.

Phosphates, likewise, were found to be increased to an average of about 17 mg. per cent compared to the normal of 5 to 6 per cent. They observed that the fragility of cells increased.

(10) Fragility of Red Blood Cells

Bacharov (1936) went into the question of the fragility more completely and concluded that every hour the blood remains in the body after death increased the fragility of the cells about 0.02 to 0.04 and equals approximately two to three days of conservation in a refrigerator. His reactions dropped from 25 per cent to 5 per cent when only bloods which had spontaneously "disagulated" were used (14).

Other investigators have reported similar results with cadaver blood but their findings have added nothing of outstanding merit to the fundamental work of Shamov and Yudin. This odd operation intrigued men all over the world and stimulated investigations in the conservation of fresh donor blood, placental blood, and that from patients on whom therapeutic phlebotomies are done. The immediate precursor of the voluntary donor "blood bank" was the practice of storing cadaver blood.

CHAPTER II

Part VII

Placental Blood

(1) Origin of Its Use

The earliest reference to the use of placental blood for transfusions seems to be the short article by M. S. Malinovsky which appeared in Soviet Surgery in 1934 (274).

In October 1936, Bruskin and Farberova of the Central Oncologic Institute of Moscow reported one hundred fourteen transfusions of placental blood preserved six to ten days. Their clinical work which began in June, 1936, was preceded by trials on animals and laboratory investigations of the toxic and immuno-biologic properties of such blood. They were able to retrieve 50 to 120 cc. of blood from each placenta and found an average hemoglobin value of 90 to 120 per cent, erythrocyte count of 5,000,000 to 6,000,000, and white blood count of 16,000 to 18,000. Citrate was the anticoagulant of choice and reactions were few (64).

The following year Stavskaya reported a series of cases from the Mother and Child Institute of Kiev. He collected 80 to 300 cc. of blood from each placenta, used 4 to 6 per cent sodium citrate as the anticoagulant and stored it for fifteen days. Several observations of his are worth noting.

He stated that the blood group of the child always corresponds to the mother. This we know from the work of Smith (1928) and others (142, 371) is not the case. During the first few weeks of postmortal life whatever agglutinins are present at birth diminish and disappear during the first ten days of life, after which time new agglutinins appear. This is interpreted to mean that the maternal agglutinins are lost and the infant produces its own.

It is true that no newborn child possesses agglutinins or hemolysins that act on the mother. Whatever agglutinins the child possesses at birth were derived from the mother by filtration through the placenta and no exceptions have been found to this rule (76, 182, 325).

The blood groups, however, are in various stages of development at birth. Definite agglutinogens can be demonstrated in the red blood cells of a fetus at a very early age (321). While it is true that agglutinins may not be present at birth, it is difficult to tell just when they have developed. Stavskaya's observation that placental blood like certain types of cadaver blood may be kept without preservative for ten to twelve days seems to have experimental foundation in the later observations of Kato and Poncher (1940) who showed that the average prothrombin time of one hundred newborn babies was 43.2 seconds, gradually shortening to 25 seconds by the tenth day. This suggests that the

lack of coagulation is due to prothrombin deficiency. Stavskaya also reported markedly increased potassium and calcium values for serum, demonstrated that the low level of protein was due to the globulin fraction, and that the blood was rich in estrogenic, gonedotropic, and an epinephrine-like substance.

In January 1936, Goodall, Anderson, Altimas, and MacPhail published a preliminary report of its use in St. Mary's Hospital in Montreal (143). It is this work, more than any other, which has been responsible for the widespread investigation of this source of blood since that time (136, 151, 159, 160, 299).

Knowing that other workers had been to Doctor Goodall's clinic as early as 1936 to learn the technique of retrieving placental bloods, we wrote to him concerning the origin of the idea. A part of his answer written from Montreal on July 7, 1939, is of historical significance and is recorded here: *"I really do not know who has precedence in the matter of placental blood used in transfusions. My work was discussed among my colleagues for a year before anything active was undertaken in the matter of preservation when I got information upon the preservation of the blood of cadavers as used in the Moscow School of Haematology. At that time, we were under the impression, my colleagues and I, that blood

groups were not fixed at birth, and that, therefore, newborns should be in the position of universal donors. We were disappointed to find that this was wrong and that groups were fixed at birth. All this took time. Our written work began in 1933-1934 and our work covering 300 transfusions had gone to print after correction of galley proofs before we learned that the Russians had been working upon the same problem synchronously. I do not know who has priority, and, I am sure I speak for my colleagues when I say that is a matter of small moment to us. The chief thing is that this work has advanced our knowledge of transfusion and given us an additional source of blood."

(2) Advantages

Goodall and his associates list four advantages of preserved placental blood over preserved donor blood. They are greater number of red blood cells and higher hemoglobin content, 20 to 35 per cent more coagulation power than that of adult blood, no allergic reactions due to food, and total reactions less. They recommend as a preservative the I. H. T. solution which is made up as follows: sodium chloride 7.0 grams, sodium citrate 5.0 grams, potassium chloride 0.2 grams, and magnesium sulfate 0.004 grams in 1000 cc. of distilled water.

(3) Technique

"When the baby is born it is laid upon the mother's abdomen. The cord is tied or clamped and wiped clean with a spong moistened with 75 per cent alcohol. The cord is stripped free of blood for about six inches, by the left finger and thumb armed with a sponge. The cord is cut with sterile scissors, the severed end lowered to below the vulva. A special towel with a two inch hole in its middle is put over the hand, holding the cord in such a manner that the hand is opposite the hole. The cord is passed through the aperture in the towel, and so placed that the end hangs in the funnel of the receptacle held by the nurse. Pressure on the cord is then released completely and the blood is collected." (143).

(4) Preservatives

Gwynn and Alsever (159) found isotonic sodium citrate a better preservative than the Russian I. H. T. solution recommended by Goodall and suggested glucose be added to make at least a 2 per cent final mixture, for they found that only when quantities greater than 2 grams of glucose were added to each flask of placental blood did the maximun delay in the development of hemolysis occur. This quantity amounted to about a 1 per cent solution of glucose in the total volume.

They reported that both red and white cells remain normal for three months. This may be true for the red blood cells but is contrary to all other reported data for white blood cells in donor and cadaver bloods.

To prove that the mother was not harmed by the procedure two series of eighty-six cases each were studied in relation to the elapsed time of the third stage of labor. The cases where the cord and placenta were allowed to remain intact required eight minutes and five seconds. The cases where the cord and placenta were emptied of their blood took eight minutes and five and six-tenths seconds.

They feel that storage for two to three months is safe and effective.

(5) Massive Transfusions

Kantorovich in 1938 advocated placental blood for massive transfusions and Soloverov and Chernomardik (1937) have reported their experiences with the mass production of isohemoagglutinating serum from such blood.

This new source of therapeutic aid is in its first stage and a few pains undoubtedly are to be expected. As a whole, however, the results with placental blood have been favorable.

CHAPTER II

Part VIII

Donor Blood and the "Blood Bank" Concept

Neither the vision of having blood on hand for emergencies nor the attempt to keep it long enough to make transfusion an elective operation are very new. Landois (1873) preserved defibrinated blood for many hours. He tells us that Folli in 1849 had preserved defibrinated blood for at least thirty-three hours and then used it successfully, Hédon in 1902 separated the cells from the plasma and resuspended the cells at a later date for transfusion.

Weil was the first investigator to store blood from three to five days before giving it with assurance that all was well. Lewisohn (1915) advised against keeping such blood for over twelve to twenty-four hours. To Weil must go the credit of first purposely giving preserved, whole, stabilized blood. Hédon (1917), using citrate and glucose as Hustin had done in 1914, was able to preserve animal cells for a month and then use them with impunity. Collection of fresh human blood in large quantities was a later development.

The first article using the term "conserved" blood in the present sense of usage was published by D. N. Belensky of the International Clinic of the Regents of Moscow in 1928 under the title "A Simple Method for the Transfusion of

Conserved Blood." He followed it with studies in 1930 and in 1935 summarized his early work with the conclusion that conserved blood undergoes in vitro biochemical and morphologic alterations of a destructive character, terminating eventually in complete loss of its functional characteristics. It should be considered, therefore, not as true blood but as a solution containing most the the components of blood. He found the clinical results excellent. Comparative studies between fresh and conserved blood showed the latter to be inferior when over twelve days old, but noted that post-transfusion reactions in cases of severe anemia and shock were fewer with blood of two to three days storage than with fresh blood.

The interest aroused by the cadaver blood transfusions of Shamov (1929) gave the impetus which resulted in the "blood bank."

In the report of the work of the Third Congress of Russian Physiologists (1929) there were several articles showing this awakened interest in the subject. Among them were: "Coagulation and Stabilization of Blood Proteins," Utilization of Bayer 205 for Transfusions of Stabilized Blood," and "About Certain Elements which Coagulate and Stabilize Blood in Vitro and in Vivo by S. S. Brjunchonenko and associates (228).

Soon afterwards reports started to appear from many

sources. From the Central Institute of Hematology and Transfusions of Moscow under the directorship of Professor A. Bagdassarov a continuous series of investigations have been carried on since that time. They began with the experimental work of Perelman (1931) sho showed that blood kept in physiological citrate solutions changes more slowly at 4° than at room temperature (315). Arutiunian (1932) confirmed clinically Perelman's earlier experimental work by giving transfusions of stored blood from voluntary donors to other individuals. Balachovsky (1932) and his associates recommended the solution of sodium citrate, sodium chloride, magnesium sulfate, and potassium chloride which became widely known as the Russian or I. H. T. solution. They felt at that time that this solution was superior to isotonic citrate or a citrate glucose mixture (18).

Vlados, an associate of Balachovsky, suggested that 6 per cent citrate solutions were more effective than the physiological solution previously used (17). By 1936 Bagdassarov reported the results of 6,345 transfusions; 3,304 done at the Institute itself and 3,041 at various stations in Moscow. Among these 2,790 had been preserved with 6 per cent citrate; 2,074 with I. H. T. serum; and 1,481 with other preservatives including a few of defibrinated bloods. When the original prejudice of Balachovsky had been overruled by experimental evidence showing that glucose is an excellent

medium for erythrocytes and the resulting lactic acid formation is not toxic, glucose-citrate solutions were introduced as preservatives.

About June, 1932, conservation was begun at the Leningrad Institute of Blood Transfusion under the directorship of Doctor Yesse. By 1935 Filatov and Depp could report 1,529 transfusions with "canned" blood. From their central station blood was being supplied at this time to forty-one hospitals in the vicinity of Leningrad (125).

In March, 1934, Tencoñ and Pellazzo reported from Buenos Aires their results on the first forty-one transfusions of preserved blood in South America.

Jeanneney and Viéroz, writing in the Bulletin of the Société Nationale de Chirurgie in December, 1934, attribute the priority of this method to the South American investigators. This, of course, is not so as the preceding notations clearly show. Jeanneney stated that he did not know of previous work on the storage of blood from living donors, but that in experimenting on the dog to check Yudin's results the idea suggested itself and their first observations were reported in May, 1934.

Investigations in Poland were begun in 1933 and a series of papers by Sokolowski, Laskowski, Marat, and Alexandrowitz gives an account of their findings (140).

Tzanck, long a great student of the problem of trans-

fusions, was late in condoning the use of banked blood but his investigations stimulated perhaps by his skepticism were instrumental in accurately establishing many of the changes in preserved blood (391).

This skepticism was perhaps merited for in Karavanov's report from Shamov's clinic in 1935, where blood only seven days old had been used, there were 67 per cent reactions, 25 per cent of which were severe. Filatov (1935) reported reactions in 50 per cent and four deaths in the first six hundred fifty-nine transfusions, while Grozdov (1934) had reactions in 41.2 per cent of one hundred forty-two transfusions. As in many new departures, results were not too good at first. Bagdassarov in 1937 reported his results in over six thousand transfusions and noted reactions in 62 per cent when I. H. T. serum was used, 62 per cent with glucose-citrate solution, 65 per cent with 6 per cent citrate, and 89 per cent with 3.8 per cent citrate.

Gnoinski (140) director of the Biological and Hematological Laboratory of the Red Cross Hospital at Varsovie gave preserved blood its severest test. In 1938, after man dog experiments, he gave blood which had been preserved in 6 per cent sodium citrate for 66, 64, 66, 69, 66, 64, 66, and 64 days respectively. In each case there was a sharp rise in temperature; in most, severe chills; in four, increased urobilinogen; in four,

hemoglobinuria; and in three, severe pain with dypsnea. All recovered and all showed improvement in the blood picture. The average amount of blood given was 280 cc. From this experience he concluded that preserved blood was innocuous and of great therapeutic value. He, apparently, was not easily perturbed by temperatures of 104 to 105° for in one case in which the patient was given blood over eighty days old, he made the note that all went well,-the temperature began to fall in eight hours and was quite normal in two days.

On August 119, 1936, Durán Jordá organized for the Republican Army of Spain, under the name of the Barcelona Blood Transfusion Service, the best system of collection and distribution of blood yet devised. The service at one time had immediate access to 28,900 donors and distributed over 9,000 liters of blood before Franco's victory. The hermetically sealed containers were of an advanced design and contained blood from a pool of similar types under a pressure of two atmospheres. He used a citrate-glucose mixture (1 per cent glucose, 4 per cent sodium citrate). The blood ready for use was delivered to the front in refrigerated trucks or train cars.

In America during the years 1925 to 1935, while many individuals undoubtedly took blood from donors and saved it until sometime later before giving the transfusion, no

organized storage and distribution was noted until about 1937. In the survey of "Blood Transfusion in America" by Levine and Katzin which appeared in 1938 no mention is made of preserved blood in any form. This work was done under the auspices of the Blood Betterment Association of New York City by means of a questionnaire to which three hundred fifty hospitals responded.

In March, 1937, Fantus instituted at the Cook County Hospital in Chicago a system built around the principle of having a central depot in the hospital where donors could be sent to have blood drawn and stored for future use. He called this system a "blood bank" and in his original report stated: "Just as one cannot draw money from a bank unless one has deposited some, so the blood preservation department cannot supply blood unless as much comes in as goes out. The term "Blood Bank" is not a mere metaphor." by the end of its second year of operation, the original bank, whose long background has been traced, had handled over four thousand transfusions. During this period there were three deaths due to the procedure. In the first year there were 12.2 per cent reactions. For the second year the reactions were roughly 6 per cent. In 1939, Doctor Schirmer, full time director of the bank, reported at the Round Table Discussion of the American College of Surgeons that the total had reached over

eight thousand transfusions, that there had been no deaths in the last four thousand, and reactions had been reduced to 1.8 per cent. Just how remarkable a stride this represents can be appreciated when it is remembered that in 1926 when the question of facilitating blood transfusions came up in Leningrad a record of only forty-two transfusions could be found. This was in the institution which in a few years was to become the world renowned Institute of Blood Transfusions (126).

In the slow, simetimes staggering, rise of the operation from the stagnant pool of humoral pathology to an accredited place in the rapidly moving stream of modern medicine, it has come to a place where evolution may approach revolutionary proportions. Transfusions carried by this new found power, the blood bank, may be overdone; blood has lost not only the awe it held for the ancients but perhaps some of the respect it deserves from the moderns. Its limitations must be recognized, its indications crystallized, and its indiscriminate use condemned. Already many studies have been made and more are being made to more clearly define the changed properties of this living tissue, in respect to the time it has remained outside the body and its effect on re-introduction. To investigate some of these properties is the purpose of the studies to follow.

CHAPTER III

REPORTED CHANGES IN PRESERVED BLOOD

Part I

Biochemical Changes

(1) Hemolysis

The most obvious change in preserved blood is the gradual hemolysis that takes place. Any change in the external medium of the cell is likely to alter the cell membrane. Stewart (1909) offered both histological and physicochemical evidence to show that alterations of the superficial layer of the envelope play an important, often decisive, part in regulating the exchange between the corpuscles and plasma. He felt that the native blood pigment probably is present in combination with the stroma constituents in the form of a gel. When water passes into the erythrocyte through the altered envelope, the hemochrome gel is transformed into an aqueous solution of hemoglobin. This aqueous hemoglobin insofar as the cell is concerned is a foreign body and as such is extruded. The envelope is pigment free and has no affinity for the blood pigment. This hemochromolysis, the change of hemochrome into hemoglobin, may be distinguished from stromatolysis. The former is accompanied by a relatively small, the latter by a

relatively large escape of electrolytes from the corpuscles. To Stewart there seemed evidence enough to suggest that the electrolytes which escape may be divided into three fractions: a portion presumably in solution, which escapes with even the gentlest methods of laking; a portion, hypothetically in loose combination or adsorbed, set free only by more energetic methods; and a portion, more intimately a part of the cell structure, which is freed only by such strong action as incineration.

This work was one of the earlier, better attempts to critically analyze a phenomenon which had long been observed. Observations in this laboratory indicate that there need not be any relation between hemoglobin loss and electrolyte loss from the cells (Chapter IV).

The toxicity of hemolyzed blood has been ascribed to many different factors. Amberson (1937) in a most complete summary of the subject reviews what have been thought to be the chief etiological factors in this toxicity. They are:

(1) Stroma as Toxic Agent.

Beginning with Schmidt in 1875 a great number of workers have ascribed the accelerated clotting of blood upon the addition of hemolyzed blood to the stromata of the laked cells (378), while Silberman (1886) believed that it was not the stromata of the red blood cells so much as the fibrin ferment released from destroyed leucocytes(367). It has been

suggested that the whole phenomenon may be due to the mechanical blocking of the smallest blood vessels (93).

(2) Improper ion balance.

Kronecker (1882) first suggested that the toxic effect of laked blood might arise from the high potassium content of the hemolyzed cells. Brandenburg (1903) showed that bloods with cells with high concentrations of potassium such as found in the human, dog, pig, guinea pig, rabbit and horse were very toxic for the frog heart and that bloods with lower potassium contents such as the calf, sheep, goat and cat, being very low in potassium were not very toxic (55).

(3) Anaphylactic Reactions

Heidelberger and Landsteiner (1923) showed that hemoglobin is a weak antigen, hence a second injection of hemolyzed blood may give anaphylactic shock.

(4) Vasoconstrictor Action

Many authors have described a vasotonic action. Moldavan (1910) showed that whatever the toxic substance is in freshly defibrinated blood, it has a tendency to disappear, at least in part, in about one half hour. Stevens and Lee in 1884 showed that whatever the vasotonin is, it is associated with the phenomenon of clotting for there is relatively little of it in citrated plasma. Goldberger (1932) distinguishes between

a labile, rapidly disappearing substance, and a more permanent stable one; the latter derived from disintegrating cells and failing to appear if the cells are removed at once.

Amberson has noted the marked kidney damage in a hemolytic crisis following a transfusion or after massive injections of hemoglobin experimentally, when the substance collects in the form of crystals or granular casts in capsule and tubules, yet he has shown that these conditions per se are not lethal or irreversible. Since this crystallization takes place in an acid urine it is wise to alkalinize the patient or experimental animal (96). The relation between hemolysis and trauma, especially the type associated with heating of blood, has been stressed by Depp (101). Levine (253), in a concise experiment, showed that the effects on animals given overheated blood were very similar or exactly like the reactions seen after heterologous transfusions. After the injection of severely hemolyzed blood death followed as the result of vasospasm and emboli in the lung tissue. In the anuria following hemolytic crisis with spasm and plugging of the kidney vessels, Hesse and Filatov (1932) advised denervation of the kidney followed by repeated large transfusions of compatible blood.

From the gathered writings of many authors this may

be concluded; that there is a toxic substance in hemolyzed blood, therefore, hemolyzed blood should not be used for transfusions, but apparently that toxic substance is not hemoglobin.

(2) Glycolysis in Preserved Blood.

Evans (1922) showed that there is rapid decomposition of glucose with the formation of lactic acid in shed blood. This has a marked effect on the blood in two ways: (1) the pH goes down, the blood becoming more acid; and (2) the CO_2 tension goes up as the process continues.

This observation has been confirmed in the studies on preserved blood by many authors including Bagdassarov (16), Gnoinski (140), Jenneney and Vievoz (199) and Jorda and Diez (208), each of whom has stressed the fact that a glucose solution is a particularly good medium for the preservation of red blood cells. The following chart expresses at a glance the common observation that the glucose value goes down and that of lactic acid goes up.

From Bagdassarov

The study of glucolysis led Jorda and Roca to study other ferments. They found that there is no appreciable change in the urea concentration of preserved blood and concluded that the low temperatures at which the blood is stored completely destroys the ureolytic diastases. Amylase, likewise, is gradually destroyed. The presence of glucose neither retards or accelerates this process (210).

(3) Vital Capacity

Shamov and Kostriukov (1929) in their earliest experiments on cadaver blood established the fact that the vitality of the red blood cells as measured by its ability to carry on gaseous exchange remained intact as long as the cells lasted.

This work was reinvestigated and confirmed by Skudins and Barenboim (1931-1932) in Yudin's laboratory before he used cadaver blood for human transfusions.

The Leningrad Institute in an attempt to preserve the cells added hydrogen peroxide to preserved blood. This, however, was done not so much to maintain function as to prevent infection for they had tried both urotropin and the salycilates. Hemolysis from any cause, but especially infection, greatly increased the rate of oxygen consumption (16).

Ponder, in a preliminary report of his studies to the Blood Transfusion Betterment Association of New York, had

this to say: In bloods stored three weeks "no hemolysis was observed, their resistance to hypotonic saline was unchanged within the limits of experimental error and the oxygen consumption of the cells was unchanged. This last point is quite remarkable and wholly unexpected. The respiration of the red cell is a very delicate indication of its state of vitality and we had expected that it would fall off steadily, if for no other reason than that the reticulocytes mature into cells having a very much smaller oxygen consumption."

Ponder pointed out that in those cases where oxygen consumption suddenly increased enormously (10 - 100 fold) contamination of the blood by bacteria was always the cause, yet without such a fine test this condition would not have been suspected. He concluded that absence of lysis and general satisfactory appearance of the blood does not mean that it is not contaminated.

Novak (296), to guard against such contamination, has suggested the use of sulfanilamide in all preserved blood.

Ingeniously taking advantage of the fact that the rate of oxygen consumption increases with infection, Jorda (205) enclosed all blood in hermetically sealed containers under two atmospheres of pressure. The amount of oxygen in this quantity of air was sufficient to turn the blood a bright red.

If, however, the blood had been contaminated, the oxygen consumption increased so rapidly that the blood would turn dark and venous in character, thereby serving as a subtle and automatic indicator of the fitness of the specimen for use.

(4) Protein Changes and the Process of Deamination.

Bodanov, Kagan and Depp, in 1934, made rather complete studies on the proteins before and after transfusion of fresh blood. These studies were not carried far enough to establish complete values for preserved blood but the protein changes in preserved blood apparently did not vary greatly from fresh blood in their results on the protein picture of the patient. A typical protocol is as follows:

	Total Protein	Red Cell Protein	Plasma Protein	Serum Protein	Fibrin
Before transfusion	16.55	12.26	4.29	8.9	1.3
After transfusion	19.80	15.50	4.24	3.4	0.83
Increase per cent	19.6 %	19.58%	-0.3%	3.0%	-2.84

Knoll (1939) showed considerable variation of the albumen-globulin ratio with different preservatives, the inversion being marked in both heparin and vetren (another heparin preparation) in the second week with a normal 7:3 inverted in each case to 2:8. No explanation for this marked change was offered. In

citrate and in citrate glucose the change was not marked.

Conway and Cook, after preliminary studies in 1930, presented evidence in 1939 that shed blood rapidly loses ammonia if the carbon dioxide tension is not maintained at a level sufficient to prevent shift in pH. No actual studies have been reported on stored blood, but the question of deamination in preserved blood is of great interest because of the effect free ammonia has on the permeability of cell membranes and the redistribution of inorganic elements through this altered membrane. This phase will be entered into more fully in the section devoted to experiments.

(5) Changes in Refraction and Viscosity.

Jeanneney, Wangernez, Leynarie (1938) concluded after a careful series of refraction experiments that the index of refraction in the plasma of preserved blood for at least 15 days is essentially the same as that of fresh blood. The values varied between 1.3480 and 1.0366.

Knoll (1939) has reported an increase in viscosity in citrated plasma from 1.64 to 2.82 in three weeks. This persistent increase in viscosity played a decisive part in the attitude of the Moscow Hematological Institute which determined the use of their special preservative in equal parts with the blood.

(6) Redistribution of Inorganic Elements Between Plasma and Cells.

Duliere in 1931 reported a constant enrichment of plasma potassium. This work was confirmed in this laboratory in April of 1938 and later by De Gowin (1939). In July (1938) Jeanneny and Servantie reported similar findings and in October reported that as the potassium went up sodium went down.

Knoll, in a very extensive study compared the changes in solutions of sodium citrate, heparin, vetren, the Leningrad solution and the Moscos solution. There were differences in the various preservatives, glucose citrate giving the best results. These conclusions were as follows: calcium remains almost constant, potassium gradually increases, sodium gradually decreases, chlorides decrease, alkaline reserve as measured by carbon dioxide content gradually diminishes and undetermined nitrogen very slowly increases while indican decreases.

Balakhovsky and Ginzburg have reported a slow rise in plasma phosphates (17).

CHAPTER III

MORPHOLOGICAL CHANGES

Part II

(1) Erythrocytes

Red cells are well preserved whether they be in cadaver, placenta, or donor blood. There is some difference between the rates at which deterioration takes place but the reports are so uniform from the different clinics that individual statistics are of no added value (98, 198, 206, 411). In citrate there may be a loss of 1,000,000 to 1,500,000 in thirty days. Gnoinsky has divided the deterioration into five stages: (1) Stage of ondulation, (2) Stage of crenation. (3) Stelliform phase. (4) Moribund phase. (5) Ghost cells. Bagdassorov (37) has pointed out that these phases do not hold for blood in glucose for there in the early stages instead of the usual shrinking in size the cells may actually become and remain larger for awhile (16).

All agree that the addition of glucose to the preservative definitely aids in the preservation of red blood cells.

(2) Hemoglobin

There is no divergence of opinion in the observation that hemoglobin levels remain constant or are augmented slightly

by evaporation of fluid.

Popova (1934) measured the resistance of the hemoglobin by measuring the time in seconds it takes for the oxyhemoglobin spectrum to disappear after adding sodium hydroxide to the blood. For normal males the average time is 60.5 seconds, for females, 56.2 seconds. Placental blood has greater resistance than that of the mothers from which it came and preserved blood resistance is increased to 72.5 seconds or higher.

(3) Leukocytes

Unger (1921) showed that the addition of citrate to blood caused marked changes in the form and function of white cells. Duran Jorda (204) was among the first students of preserved blood to point out that the leucocytes are gradually destroyed and become amorphous masses by the fifteenth to the twentieth day. The polymorphonuclear leukocytes are the least resistant and disappear in about one week, the eosinophiles most resistant and the lymphocytes intermediate in the length of preservation of normal contour. Glucose did not affect the destruction of these cells. Tzanck and Dreyfuss (391) pointed out that the monocytes may completely disappear in from three to four hours and this observation was confirmed by Gnoinski (140). Kolmer (230) reported obvious changes in the leucocytes in twenty-four hours. In our laboratory these

changes were observed before any literature had been seen.

(4) Plateletes

Drinker and Brittingham (1919) felt that one of the chief causes of reactions following transfusions with citrated blood was the destruction of the platelets with the liberation of toxic substances. All subsequent investigators have concured in the observation but not the conclusion. By the end of a week, the platelets have fallen to levels between 10,000 and 50,000 and in some preservatives have completely disappeared.

(5) Fragility of Erythrocytes

The red cells become more fragile as they grow older. Depp (1934) reported that for the first two days the fragility remains unchanged, approximately 0.44 for partial hemolysis and 0.24 for complete. From the third day, there is an increase which by the sixth day reaches 0.6, the seventh day 0.76-0.80, the tenth day 0.85, and on the eleventh and twelfth day the first signs of spontaneous hemolysis begins to show in blood stored in citrate. The addition of glucose retards this hemolysis to about the thirty-fifth day. Both shaking and warming increase the fragility.

All subsequent observers have simply confirmed these findings, the results varying but little from country to country.

(6) Prothrombin

While prothrombin in the strictest sense is not a morphological element, it is included here as a convenience. Results concerning its changes have not been as consistent as those for the countable elements.

Quick, Stanley Brown and Bancroft in 1935 showed that this substance or quality is the precursor of thrombin, the active coagulating enzyme; and that it is remarkably constant in fresh normal blood. Brinkhouse, Smith and Warner (1937) showed that serious hemorrhage does not occur as a result of prothrombin deficiency until the level sinks below 20 per cent.

Rhoades and Panzer in 1939 reported a fall of prothrombin to ineffectual levels within a week after storing preserved blood. These investigators used the method of Quick in determining their levels.

In December, 1939, Lord and Pastore using the Brinkhouse, Smith and Warner method reported that bank blood is an adequate source of plasma prothrombin for about nine days and reaches 61 per cent of normal by the end of the third week.

They were unable to account for the difference between their results and those determined by the Quick method, but did stress the importance of good refrigeration as a large factor in preserving the prothrombin content.

(7) Sedimentation rate

Jeanneney and Vieroz (1934) determined that the sedimentation rate of preserved cells gradually decreases with age. At twenty days it may have difficulty in flowing. Fourestier and Paillas (132) state that a rapid sedimentation rate in the donor is perhaps a frequent cause of post-transfusion reactions. At the Cook County Blood Bank it is regarded as one of the most important findings in rejecting a donor (287). Of particular interest is the theory presented by Jeanneny, in which he postulates that sedimentation rate changes and perhaps some reactions are due to the electrical charge on the cells; e. g. assuming all the charges on the cells are positive, trauma in any form might change the charge on the injured cells to negative, then instead of a mutually repellant force of like charges in the suspension, there would be attractions of cells leading to clumping in the form of pseudo-agglutination or even true agglutination, with all of the clinical sequellae consequent to such a condition (132).

(8) Length of life of Erythrocytes

Asby (in 1919), after a thorough review of the literature, decided that the published statistics on the length of life of transfused erythrocytes was too short and the methods used to determine them inaccurate. She attempted to get more accurate results by transfusing a patient with blood of a group other

than his own, then by treating specimens of this patient's blood with serum that agglutinated his cells, leaving the cells of the transfused blood intact, she could count the unagglutinated cells and trace the time of their disappearance. She concluded that the life of a fresh donor's erythrocyte in the body of the transfused lives at least thirty days, at times one hundred and ten days.

Martinet (1938) stimulated to check the findings of Ashby, after the severe criticism of Görl (1926), by means of the agglutinogens M and N, determined that fresh transfused cells remained in the recipient's cells at least sixty days.

He determined this by giving, for instance, the blood of an ON donor to an OMN recipient, then collected two tubes of blood. To one (1) he added anti-M serum and to the other (2) anti-N serum. In tube (1) the cells of the recipient containing MN are agglutinated, while the donor's cells containing only N isoagglutinogen remain suspended in the serum and give it a cloudy appearance. The end point is reached at the time at which the serum becomes clear, indicating the final destruction of all the donor's cells. In tube (2) since both donor and recipient cells are acted on by anti-N serum both are agglutinated and the serum becomes limpid at once. The test lies in following, in this case, the degree and final disappearance of the cloudiness in tube (1).

Philip Levine, using this method, was able to report at the American College of Surgeons meeting in Philadelphia, October, 1939, preliminary studies (to be published later) on preserved blood of group M transfused into group N individuals with the following results:

Fresh blood	95+	days
Three day old blood	80	days
Ten day old blood	60	days
Fourteen days old blood	20	days

CHAPTER III

Part III

Changes in Immune Properties

(1) Complement

Early opponents of citrated blood transfusion (Unger 1921) advanced the objection that sodium citrate destroys the complement. Kolmer 1939 states: "This, however, does not appear to be true, indeed the reverse appears to be the case as sodium citrate in 0.35 per cent concentration apparently preserves the complement of human blood in a remarkable degree, analogous to the preservation of guinea pig complement of the Wasserman reaction by sodium acetate and sodium chloride."

He did find, however, that at the end of 7 - 21 days there was a loss of bactericidal activity for one or more of three test organisms. Whether this was due to gradual inactivation of complement and opsonins, loss of bacteriolysins and other none specific leukins or plakins was not determined.

(2) Phagocytosis

Phagocytic activity was rather markedly reduced in seventy-two hours and there was almost total absence of phagocytosis on and after the seventh day. Here again

Kolmer felt: "the marked changes in the opsonophagocytic activities may have been due in part to deterioration of opsonins but undoubtedly were mainly and most likely entirely due to autolytic changes in the neutrophiles."

Jorda and Llorach (209) found that preserved blood lost half of its complement by the seventh day and by the twentieth is almost completely destroyed, the loss paralleling fairly accurately the loss of white cells.

Jeanneney, Castanet, and Cator (June, 1939) have made an attempt to measure accurately the hemobacteriacidal power of the blood. By preparing dilutions of the organism against which the blood is to be tested of from 1,000 to 25,000,000 per cc. and adding to each an equal quantity of blood, hanging drops may be made from a gelatine bouillon after twenty-four hours which will show the bactericidal power in each dilution. This may be recorded in terms of percentage and used as an index from day to day in following the course of a patient. Using this same test he has demonstrated the gradual fall in the bacteriacidal power of preserved blood.

CHAPTER IV

EXPERIMENTAL STUDIES IN BLOOD PRESERVATION

Part I

Repartition of Potassium in Cells and Plasma

It has been the common observation of all investigators of blood preservation that if the blood is kept long enough it invariably becomes hemolyzed or laked (chapter III). From the earliest transfusions (Denis, 1667) severe and fatal reactions have been associated in an ill-defined but constant manner with hemolysis in the blood stream of the recipient; since the use of preserved blood has become more widespread, the degree of hemolysis observed in the donor's blood has been used as a rough index of the suitability of such blood for transfusion (121, 142, 411)

Naunyn in 1868 first described the toxic properties of laked blood. His observations have been reaffirmed by many subsequent investigators beginning with Landois, but the nature of the toxic substance or substances has yet to be revealed. The purpose of the experiments that follow is to throw new light, if possible, on the nature of this substance.

Phemister and Handy reported in 1927 that there is something in slightly laked bloods which causes vasodilatation while more severely traumatized bloods cause vasoconstriction.

It is not histamine, the product of adrenaline disintegration, nor the pituitary principle. It is not developed as the result of changes in oxygen, carbon dioxide, or hydrogen ion concentration; shifts in temperature; or exposure to light or air. Petroff observed vasoconstriction of the splenic, renal, and pulmonary vessels following the injection of hemolyzed blood, but did not isolate the causative factors (319). Amberson has established the fact that the toxic factor is not hemoglobin (5).

Kronecker as long ago as 1882 suggested that the toxic effects of laked blood were due to its high potassium content. The report of Dulière, in 1931, of a constant enrichment of the serum potassium in whole blood stored for several days raises again the question of the relation of this toxic substance to the unfavorable results still encountered too frequently in the operation of blood transfusion. The cells of a healthy human blood in vivo contain twenty times as much potassium as the plasma. Any dislocation from its natural environment introduces the possibility of altering this normal distribution of the chief base of the cells. Altered structure is tantamount to altered function. Banked blood, therefore, seems worthy of reinvestigation from the point of view of its altered chemical composition.

First Series

The first group of experiments was designed to check Dulière's statement. Two samples of the same human venous blood were collected with aseptic precautions and kept in pyrex flasks stoppered with cotton, in the dark, in a refrigerator at 4° C. in the following manner.

Experiment 1

The blood, 250 cc., was kept under liquid petroletum.

Experiment 2

The blood, 250 cc., was mixed with 2.5 per cent solution of sodium citrate in sufficient quantity to make a mixture containing 0.31 Gm. per 100 cc. of blood.

At the same time a 5 cc. sample was mixed with heparin (Connaught) in a centrifuge tube and spun for one hour. This served as the sample for base line determinations.

Portions of the serum or plasma were pipetted off at twenty-four hour intervals for five days and then at approximately weekly intervals for a month. With each analysis, material for culture was taken and streaked on blood agar plates. These were observed for growth at the end of twenty-four and forty-eight hours.

Methods

Potassium determinations were done throughout by the argenticobaltinitrite method of Kramer and Tisdall (231)

as modified by Breh and Gaebler (57) and further refined by Truszkowski and Zwemer in 1936. The final readings were made on the Evely photoelectric colorimeter (117). The values given are the mean of two aliquots. Cell volume was determined by Sanford-McGath tubes (343), spun for one hour at 2,000 revolutions per minute. Specific gravity was measured by the method of Barbour and Hamilton (19). The plasma protein content was calculated from the specific gravity by the formula of Weech, Reeves, and Goettsch (416).

Results

Blood Kept Under Oil Without A Preservative

The blood of the donor, Doctor J. S., was of group B.

Procedure:

In addition to the determinations made at the time of the bleeding which served as basic values, eight other sets of analyses were made, and the results are expressed in four ways (table 1):

1. Column 4; actually observed; serum potassium as milligrams per hundred cubic centimeters of serum.

2. Column 5; by calculation as milligrams of potassium in the serum of 100 cc. of blood. This figure for practical purposes gives at a glance the actual amount of serum potassium in every hundred cubic centimeters of blood on any given day when preserved in the stated manner.

3. Column 6; by calculation as milligrams of potassium

given off into the serum by each hundred cubic centimeters of cells. This figure is theoretically more convenient for comparative purposes, as it obviates the differences created by bloods whose cell volumes may vary markedly from normal.

4. Column 7; as percentage of potassium which has diffused out of the cells.

Each day's values represent the sum of the increments for that particular day, care being taken throughout to estimate the amount of potassium removed in the various test samples.

Basic Value:

Hematocrit reading	54.3 per cent Cells
	45.7 per cent Serum
Plasma potassium	21.0 mg. per cent
Whole blood potassium	202.0 mg. per cent
Cell potassium (calculated)	354.0 mg. per cent
Plasma specific gravity	1.0274
Plasma proteins	6.87 Gm. per cent

Calculation: (a) For base line or zero values

Blood sample	250.0 cc.
Serum volume 250 x .457 (per cent serum) =	114.4 cc.
Determined serum potassium	21.0 mg.per cent
Total potassium in original serum	24.0 mg.

(b) For first day's increment.

2 cc. sample taken.	
Serum potassium (observed value)	40.0 mg.per cent
Potassium in 2 cc. sample	0.8 mg.
Potassium in residual serum 114.4-2x.4 =	45.0 mg.
Total potassium in serum after 24 hours 45 + .8 =	45.8 mg.
Increase in total potassium in serum in 24 hours	
45.8 - 24.0 (originally present) =	21.8 mg.

Increase in serum potassium of 100 cc. blood in first 24 hours $21.8 \times \frac{100}{250 (cc.)} = 8.7$ mg.

Amount of potassium given off in first 24 hours by each 100 cc. cells

$$21.8 \times \frac{100}{250 \text{ (cc. blood)} \times .543 \text{ (\% cells)}} = 16.0 \text{ mg.}$$

Percentage of cell potassium diffused out into serum in first 24 hours

$$\frac{16 (\text{amount lost})}{354 (\text{original cell potassium})} = 4.5$$

In a similar manner each day's results were determined and expressed as the sum of the increments.

The results are shown in table 1 and figure 1.

Experiment 2: The same steps were repeated in experiment (2) and the results are graphically represented in figure 1.

In each of the two flask there was a steady rise in serum or plasma potassium. In the hematocrit tube, however, for the few days observed, the increment was less and suggested either heparin was a markedly superior preservative or the slow diffusion was the result of some other factor.

In each instance, there was discernable discoloration of the supernatant fluid by the fifth day and obvious hemolysis by the fourteenth.

Discussion

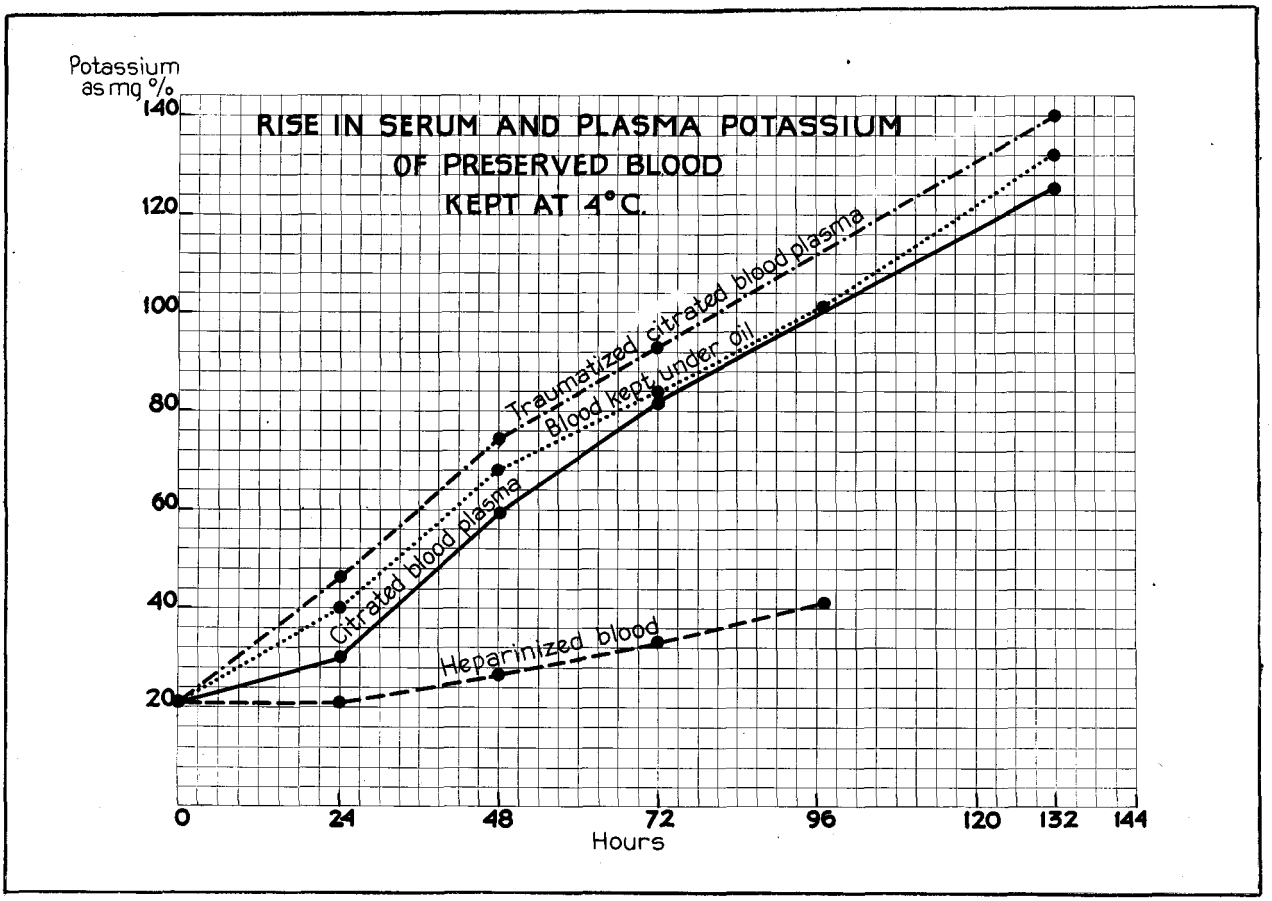
The findings in table 1 suggest that blood kept under oil at a constant temperature loses in the first week at least 25 per cent of its cell potassium, at the end of three weeks about 40 per cent and diminishing quantities from that

Table 1
Experiment 1
Blood Kept Under Oil Without Preservative

1	2	3	4	5	6	7	8
Date	Sample	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Hemolysis (Observed)
			Observed Value Per 100 Cc. of Serum*	Sum of Increments Per 100 Cc. of Blood	Sum of Increments From 100 Cc. of Cells		
3/27/38	1	1	40.0	8.7	16.0	4.5	0
3/28/38	2	2	68.6	21.1	39.2	11.0	0
3/29/38	3	3	83.0	27.2	51.0	14.2	0
3/30/38	4	4	100.8	35.1	64.7	18.3	0
3/31/38	5	5	133.2	48.6	89.7	25.3	0
4/ 9/38	6	14	200.0	76.1	140.5	39.6	++++
4/15/38	7	20	206.4	78.6	145.2	40.9	++++
4/29/38	8	34	225.0	86.0	158.0	44.8	++++

*Values uncorrected for sample removed.

FIGURE 1



Data from experiment 1, table 1

Heparinized blood kept in centrifuge tube. The other bloods were stored in Erlenmeyer flasks.

time on. Stated in more practical terms, such blood at the end of the first week contains in the serum of each hundred cubic centimeters of blood at least 50 mg. of potassium and at any time after two weeks at least 75 mg., the amount gradually increasing

Second Series

The second group of experiments was designed: (1) to check the results of the first series, (2) to ascertain the effect of trauma (such as shaking) on the rate of loss of potassium from cells, and (3) to see what effect the shape of the container had on the rate of diffusion.

The blood of the donor, Doctor G. S., was of group O. Five hundred cc. of blood was collected and placed in three pyrex flasks.

Experiment 3

The blood, 150 cc. was kept under oil without an anti-coagulant.

Experiment 4

The blood was placed in a Sanford-Magath hematocrit tube containing heparin and spun for an hour.

Experiment 5

The blood, 150 cc. was mixed with 50 mg. of heparin.

Experiments 6 and 6B

The blood, 200 cc., was mixed with the usual sodium citrate solution.

These flasks were treated in a manner similar to the first set. The results are shown in table 2.

Discussion

From the observed values, none of the anticoagulants prevented the loss of potassium from the cells. The rate of diffusion appeared a little slower in the citrated blood. There was a distinct difference in the heparinized bloods (figure 2), that in the container with the larger interface showing the more rapid diffusion. This indicates that the shape of the container and not the anticoagulant was probably the cause of the slow diffusion in the heparinized blood in the first series. Here again, agitation by vigorous shaking hastened the process of diffusion (figure 3).

Third Series

In the third series four samples of fresh venous whole blood were preserved in 2.5 per cent solution of sodium citrate, 3 per cent solution of sodium citrate, Peyton Rous solution (339), and the Russian citrate solution (18).

Experiment 7

Blood Preserved in 2.5 Per Cent Solution of Sodium Citrate

The blood, from a professional donor, G. W., was of group O. To 125 cc. was added 17.5 cc. of 2.5 per cent solution of sodium citrate to give a mixture containing 0.31 Gm. per hundred cubic centimeters of blood. Each day

Table 2 (Experiments 3, 4, 5, 6, and 6B)

Blood Preserved with Different Preservatives

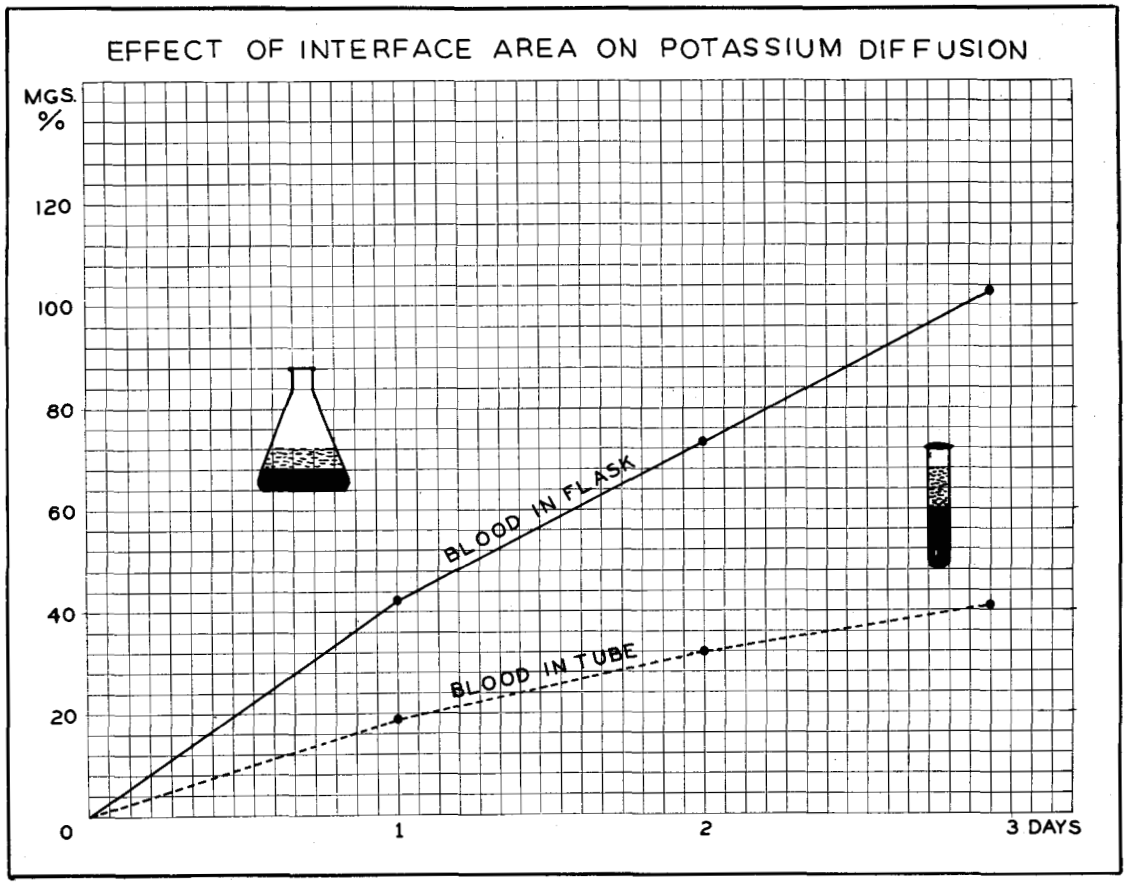
Date	Days	Observed Values of Potassium as Milligrams Per Cent*					Hemolysis (Observed	Culture (Blood Agar)
		Under Oil 150 Cc. in Flask	Heparinized 5 Cc. in Test Tube	Heparinized 150 Cc. in Flask	Citrated 200 Cc. in Flask	Citrated 200 Cc. after Shaking		
		Exper. (3)	Exper. (4)	Exper. (5)	Exper. (6)	Exper. (6B)		
5/25/38	1	42.0	19.1	43.3	29.6	42.0	0	0**
5/26/38	2	74.5	32.2	73.7	60.8	73.0	0	0
5/27/38	3	103.0	42.3	103.0	86.0	97.4	0	0
5/28/38	4	105.0	--	115.0	105.0	117.0	+	0
5/29/38	5	129.0	--	142.0	113.0	--	++	0
5/30/38	6	150.0	--	155.0	132.0	141.0	+++	0
6/ 3/38	10	174.0	--	187.0	163.0	169.0	++++	0
6/ 5/38	12	188.0	--	194.0	--	175.0	++++	0
6/ 7/38	14	182.0***	--	193.0	168.0	172.0	++++	0
6/ 8/38	15	--	--	--	183.0	--	++++	0
6/23/38	30	--	--	211.0	--	--	++++	0

* These values were determined on the samples removed from the flasks. They are not corrected for quantity, dilution or the small amount of plasma or serum removed for the analysis (2-5 cc).

** 0 = no growth.

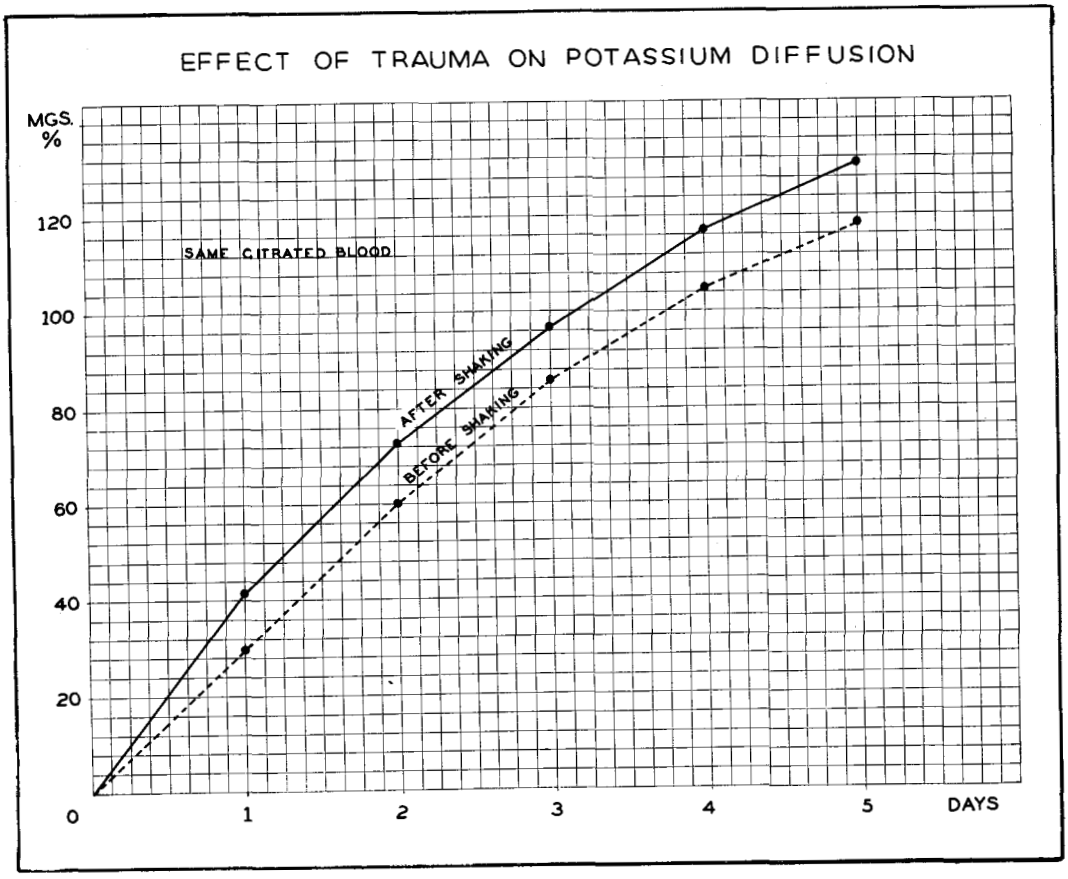
*** This decrease, as well as other terminal values in tables 3, 4, 8, 9, and 10 is unexplained.

FIGURE 2



Data from experiments 4 and 5, table 2

FIGURE 3



Data from experiments 6 and 6B, table 2

a 2 cc. sample was removed from the supernatant fluid without disturbing the cells and the potassium content determined as in experiment 1.

Basic Values:

Hematocrit reading	45.5 per cent Cells 54.5 per cent Plasma
Plasma potassium	19.8 mg. per cent
Whole blood potassium	198.0 mg. per cent
Cell potassium (calculated)	410.0 mg. per cent
Plasma specific gravity	1.0274
Plasma proteins	6.97 Gm. per cent

Calculations:

Blood sample	125.0 cc.
Plasma volume	68.0 cc.
Total fluid volume	85.5 cc.
Plasma potassium in 68.0 cc.	13.51 mg.
Theoretical potassium in total fluid	13.51 mg.
Actual potassium in sample of plasma and preservative mixture on 8/17/38 just after mixing (hereafter, the word plasma means this mixture)	12.64 mg.

The base line or zero values, therefore, are:

Plasma potassium per 100 cc.	14.8 mg.
Plasma potassium per 100 cc. of blood	10.1 mg.
Plasma potassium per 100 cc. cells	22.5 mg.

The results are tabulated in table 3 and grephically represented in figures 4 and 5.

Experiment 8

Blood Preserved in 3 Per Cent Solution of Sodium Citrate

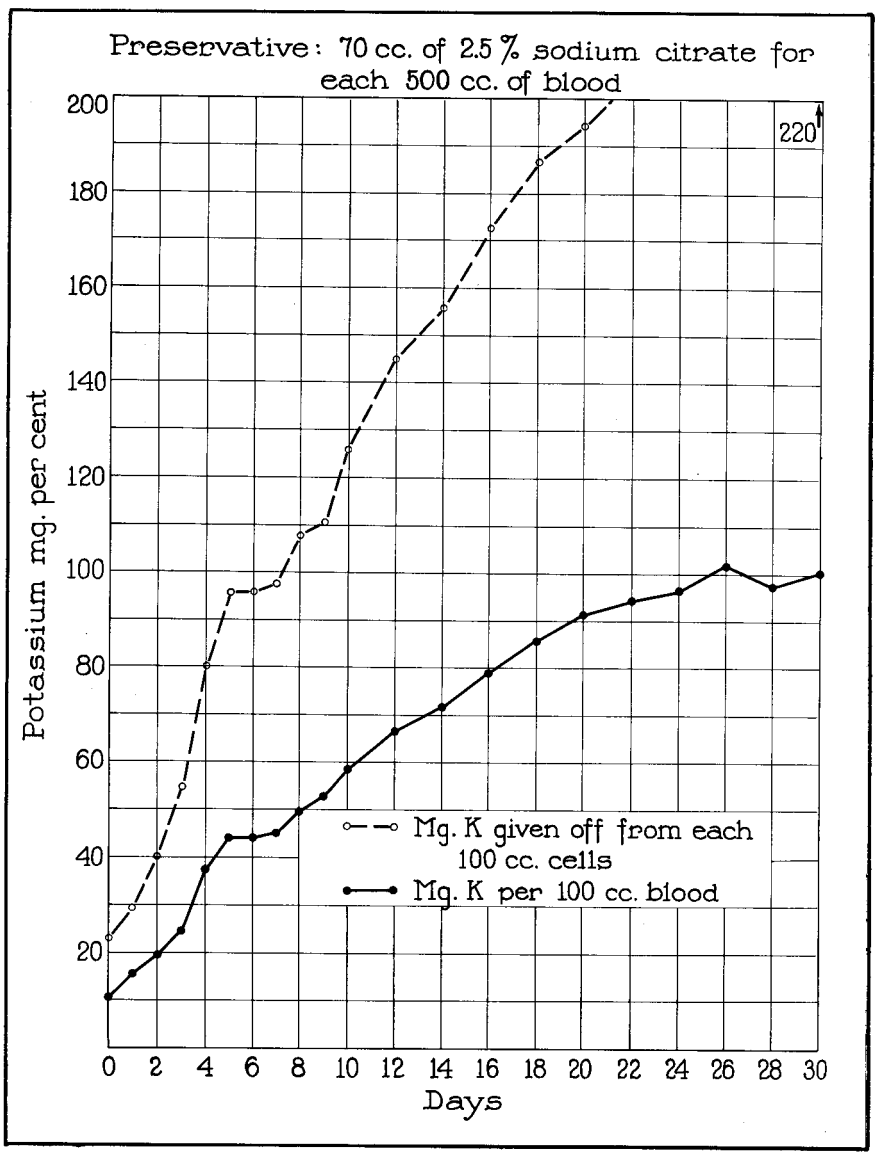
The donor was the same as in experiment 7 and the procedure was the same except that 15 cc. of a 3 per cent

Table 3
Experiment 7
Blood Preserved in 2.5 per Cent Solution of Sodium Citrate

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Hemolysis (Observed)	Culture (Blood Agar)
		Observed Value Per 100 Cc. Plasma	Sum of Increments Per 100 Cc. Blood	Sum of Increments From 100 Cc. Cells			
8/18/38	1	20.0	3.5	7.2	1.9	0	--
8/19/38	2	27.3	7.3	18.1	3.9	0	0
8/20/38	3	43.1	18.1	32.2	9.7	0	0
8/21/38	4	56.2	26.4	58.2	14.0	0	0
8/22/38	5	67.3	33.1	74.5	17.7	0	--
8/23/38	6	68.3	33.7	74.2	18.1	0	--
8/24/38	7	70.2	34.8	76.2	18.7	0	--
8/25/38	8	78.0	39.1	85.9	21.0	0	0
8/26/38	9	81.7	41.1	90.5	22.0	0	--
8/27/38	10	94.5	47.8	105.2	25.6	0	--
8/29/38	12	111.0	56.2	123.7	30.1	±	--
8/31/38	14	121.0	61.1	134.2	32.8	trace	0
9/ 2/38	16	136.4	68.5	150.9	36.7	definite	--
9/ 4/38	18	152.8	75.8	165.0	40.6	+	--
9/ 6/38	20	165.0	81.4	177.0	43.6	++	0
9/ 8/38	22	171.0	84.0	182.5	45.0	+++	--
9/10/38	24	180.0	87.7	192.0	47.0	+++	--
9/12/38	26	191.0	92.0	200.1	49.3	++++	0
9/14/38	28	182.0	88.6	192.8	47.5	++++	--
9/16/38	30	188.0	90.8	198.2	48.7	++++	--

9/20/38 pH = 7.69 ± 0.05 as determined by glass electrode.

FIGURE 4



Data from experiment 7, table 3

This chart shows the actual amount of plasma potassium in 100 cc. of blood and the amount given off by 100 cc. of cells.

solution of sodium citrate in sterile water was used as the preservative for 125 cc. of blood. The gram percentages are exactly the same, 0.31.

Basic Values:

Same as in experiment 7.

Calculations:

Blood sample	125.0 cc.
Plasma volume	68.0 cc.
Total fluid volume	83.0 cc.
Theoretical potassium in total fluid	13.51 mg.
Actual potassium in total fluid	12.64 mg.

Zero values:

Plasma potassium per 100 cc.	15.3 mg.
Plasma potassium per 100 cc. of blood	10.19 mg.
Plasma potassium per 100 cc. of cells	22.38 mg.

The results are tabulated in table 4 and graphically represented in figure 5.

Experiment 9

Blood Preserved in Russian (I.H.T.) Citrate Compound*

The blood of the donor, Doctor C. R. D., was of group O. Fifty cc. of blood was added to an equal quantity of preservative made up according to the following formula:

*Sodium chloride	7.0 Gm.
Sodium citrate	5.0 Gm.
Potassium chloride	0.2 Gm.
Magnesium sulfate	0.004 Gm.
Distilled water	1000.0 cc.

The rate of diffusion determined as in experiments 7 and 8.

Table 4
Experiment 8
Blood Preserved in 3 per Cent Solution of Sodium Citrate

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Hemolysis (Observed)	Culture (Blood Agar)
		Observed Value	Sum of Increments				
		Per 100 Cc. Plasma	Per 100 Cc. Blood	From 100 Cc. Cells			
8/18/38	1	18.7	2.2	4.8	1.2	0	--
8/19/38	2	32.8	11.1	24.5	6.0	0	0
8/20/38	3	42.1	16.9	37.1	9.1	0	0
8/21/38	4	54.6	24.3	53.3	13.0	0	--
8/22/38	5	62.6	29.0	63.8	15.5	0	--
8/23/38	6	63.0	29.3	64.3	15.7	0	--
8/24/38	7	73.5	35.0	72.0	18.8	0	0
8/25/38	8	76.2	36.5	80.2	19.6	0	--
8/26/38	9	80.1	38.5	85.5	20.6	0	--
8/27/38	10	88.3	42.7	92.1	22.9	0	--
8/31/38	14	111.0	53.6	117.6	28.7	trace	0
9/ 2/38	16	126.1	60.4	133.1	32.4	+	--
9/ 4/38	18	146.4	69.4	152.3	37.2	+	--
9/ 6/38	20	157.0	73.8	162.3	39.6	++	0
9/ 8/38	22	167.5	78.1	171.6	41.9	+++	--
9/10/38	24	172.0	79.5	174.6	42.6	+++	--
9/12/38	26	179.0	82.5	181.6	44.2	++++	0
9/14/38	28	187.0	85.5	187.8	45.8	++++	--
9/16/38	30	181.0	83.3	183.5	44.7	++++	--

9/20/38 pH = 7.76 ± 0.05 as determined by glass electrode.

Basic Values:

Hematocrit reading	48.1 per cent Cells
	51.9 per cent Plasma
Plasma potassium	15.5 mg. per cent
Whole blood potassium	196.0 mg. per cent
Cell potassium (calculated)	388.0 mg. per cent
Plasma specific gravity	1.0272
Plasma proteins	6.9 Gm. per cent

Calculations:

Blood sample	50.0 cc.
Plasma volume	26.0 cc.
Total fluid volume	76.0 cc.
Theoretical potassium in total fluid	9.88 mg.
Actual potassium in first sample	10.72 mg.
Zero values:	
Plasma potassium per 100 cc.	14.5 mg.
Plasma potassium per 100 cc. of blood	21.44 mg.
Plasma potassium per 100 cc. of cells	44.6 mg.

The results are tabulated in table 5 and graphically represented in figure 5.

Experiment 10

Blood Preserved in Peyton Rous Solution*

The blood of the donor, Doctor J. S., was of group B. To 150 cc. of blood was added 250 cc. of 5.4 per cent solution of glucose in distilled water and 100 cc. of 3.8 per cent solution of sodium citrate.* Potassium determinations were made as before.

Basic Values:

Hematocrit reading	50.0 per cent Cells
	50.0 per cent Plasma
Plasma potassium	22.8 mg. per cent
Whole blood potassium	209.0 mg. per cent
Cell potassium (calculated)	388.0 mg. per cent
Plasma specific gravity	1.0283
Plasma proteins	7.28 Gm. per cent

Table 5
Experiment 9
Blood Preserved in Russian Citrate Compound

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Hemolysis (Observed)	Culture (Blood Agar
		Observed Value Per 100 Cc. Plasma	Sum of Increments Per 100 Cc. Blood	Sum of Increments From 100 Cc. Cells			
8/21/38	1	18.5	6.0	13.5	3.2	0	0
8/22/38	2	24.3	14.5	31.2	7.8	0	-
8/23/38	3	30.2	22.7	43.2	12.8	0	-
8/24/38	4	32.9	26.3	55.7	14.1	0	0
8/25/38	5	35.6	29.8	63.8	16.0	0	-
8/26/38	6	40.3	35.9	75.5	19.2	0	-
8/27/38	7	46.1	43.0	90.5	23.1	0	-
8/31/38	11	62.6	60.4	126.7	32.4	trace	0
9/ 2/38	13	72.0	68.7	144.0	36.8	+	-
9/ 4/38	15	83.9	83.9	175.0	45.0	+	-
9/ 6/38	17	95.0	95.4	199.0	51.2	++	0
9/ 8/38	19	102.8	103.4	215.5	55.4	+++	-
9/10/38	21	111.8	111.8	233.0	60.0	+++	-
9/12/38	23	113.0	113.0	235.5	60.6	++++	0
9/14/38	25	117.0	116.4	242.5	62.4	++++	-
9/16/38	27	122.0	120.7	252.0	64.7	++++	-
9/18/38	29	123.0	121.7	254.0	65.2	++++	-

9/20/38 pH = 7.59 ±0.05 as determined by glass electrode

Calculations:

Blood sample	150.0 cc.
Plasma volume	75.0 cc.
Total fluid volume	425.0 cc.
Theoretical potassium of first sample	17.1 mg.
Actual potassium of first sample	21.2 mg.
Zero values:	
Plasma potassium per 100 cc.	5.1 mg.
Plasma potassium per 100 cc. of blood	14.2 mg.
Plasma potassium per 100 cc. of cells	28.3 mg.

The results are tabulated in table 6 and graphically represented in figure 5.

The high percentage of sodium citrate in this preservative contraindicates its use for transfusions; the cells, however, may be resuspended in saline and used.

Summary Part I

1. There is a daily increase in the amount of potassium present in the serum or plasma of whole blood kept in vitro under aseptic bacteriostatic conditions.

2. The transference of potassium from cells to plasma begins at the time of withdrawal from the blood stream, is rapid at first and gradually diminishes in rate.

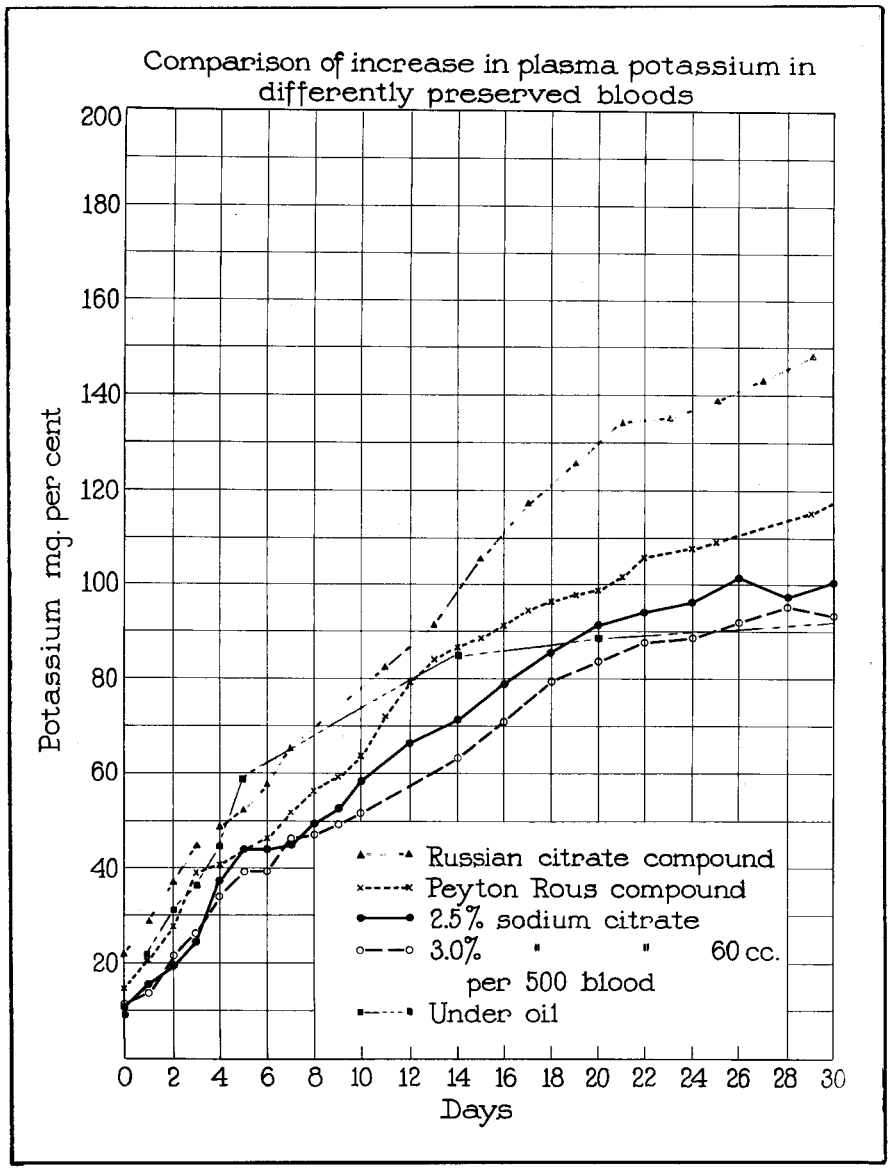
3. The total amount found in the serum at the end of ten days reaches 25 per cent of the total potassium content of the red blood cells in the fresh state and at the end of thirty days may exceed 50 per cent.

Table 6
Experiment 10
Blood Preserved in Peyton Rous Compound

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Hemolysis (Observed)	Culture (Blood Agar)
		Observed Value Per 100 Cc. Plasma	Sum of Increments Per 100 Cc. Blood	Sum of Increments From 100 Cc. Cells			
7/16/38	1	7.1	5.9	11.9	3.0	0	0
7/17/38	2	9.7	13.0	26.3	6.8	0	-
7/18/38	3	13.9	24.6	49.6	12.0	0	-
7/19/38	4	14.5	26.3	53.0	13.5	0	0
7/20/38	5	16.8	32.4	65.2	16.7	0	0
7/22/38	7	18.5	36.8	74.2	19.0	0	0
7/23/38	8	20.4	42.0	84.6	21.6	0	0
7/24/38	9	21.4	44.6	90.0	23.0	0	0
7/25/38	10	22.9	48.5	97.8	25.0	0	0
7/26/38	11	26.1	57.4	114.8	29.6	0	-
7/27/38	12	29.1	64.6	130.2	33.3	0	-
7/28/38	13	30.8	68.9	138.9	35.5	0	-
7/29/38	14	32.1	72.0	145.2	37.2	0	-
7/30/38	15	32.8	73.9	149.0	38.1	0	-
7/31/38	16	33.5	75.7	152.2	39.0	0	-
8/ 1/38	17	35.0	79.5	160.2	41.0	0	-
8/ 2/38	18	35.7	81.8	163.6	42.2	0	-
8/ 4/38	20	36.6	83.4	168.0	43.0	0	-
8/ 5/38	21	37.5	85.7	172.6	44.2	0	-
8/ 6/38	22	39.6	90.5	182.3	46.6	0	-
8/ 8/38	24	40.0	91.8	184.9	47.3	0	-
8/13/38	29	43.5	100.2	201.9	51.7	0	0
8/15/38	31	46.3	107.0	215.8	55.6	0	0
8/31/38	47	53.8	124.3	250.5	64.1	0	-
9/ 6/38	53	62.4	145.0	290.0	74.7	0	0

9/20/38 pH = 6.84 ± 0.05 as determined by glass electrode.

FIGURE 5



Data from experiments 1, 7, 8, 9, 10 and tables 1, 3, 4, 5, and 6

4. The rate at which the potassium is given up by the cells is greatly increased by shaking.

5. The rate at which potassium is given off increases as the are of interface between the cells and the supernatant serum increases; that is, the cells apparently keep better in tubular containers of small diameter than in wide bottomed flasks.

6. Hemolysis appeared at varying times in the different samples; none was observed in the sample preserved in the citrate-saline-glucose solution.

7. Changes observed in these experiments were not due to bacterial infection.

8. Sodium citrate in a 0.31 Gm. per cent solution is more effective as a preservative than the more complex Russian (I.H.T.) citrate compound.

9. The citrate-saline-glucose solution of Rous and Turner prevents loss of hemoglobin from the cells but not the loss of potassium.

CHAPTER IV

Part II

A Comparison of the Rates at which Cells Lose Potassium and Hemoglobin

In the third series of experiments repeated attempts to measure accurately the small amounts of hemoglobin in the plasma of only slightly hemolyzed bloods were unsuccessful with the usual acid hematin methods such as the Helige and Sahli. With the Pulfrich photometer, however, small daily increments were demonstrable with greater consistency. The method used is a modification of that described by Heilmeyer (173). The content of hemoglobin may be calculated in grams per 100 cc. of plasma then re-expressed as grams lost from the cells per 100 cc. of blood.

The potassium determinations were done as described in part I, the cultures on blood agar plates, and the pH determinations first by titration and then checked by means of a Helige glass electrode potentiometer which had been calibrated by buffers.

Experiment 11

Blood Preserved in Diluted Russian Citrate Compound

The blood of the professional donor, J.F, was of group O. Equal parts of blood and diluted preservative were mixed by adding 125 cc. of blood to 125 cc. of preservative

which contained 675 mg. of sodium chloride, 625 mg. of sodium citrate, 25 mg. of potassium chloride, and 0.5 mg. of magnesium sulfate (71).

The blood and preservative each were checked for initial pH values and compared to the pH of the mixture at the end of the experiment.

Hemoglobin determinations were done at the first suggestion of hemolysis. Cultures were taken at well spaced intervals.

The sero value used in determining the daily increments of hemoglobin was 40 mg. in the plasma from 100 cc. of blood. This quantity is our normal value for blood which has been centrifuged for one hour.

Basic Values:

Hematocrit reading	45.6 per cent Cells
	54.4 per cent Plasma
Plasma potassium	17.6 mg. per cent
Whole blood potassium	192.0 mg. per cent
Cell potassium (calculated)	400.0 mg. per cent
Plasma specific gravity	1.0253
Plasma proteins	8.25 Gm. per cent
Blood pH	7.3
Preservative pH	7.4

The results are tabulated in table 7.

Experiment 12

Blood Preserved in Sodium Citrate and Eschatin

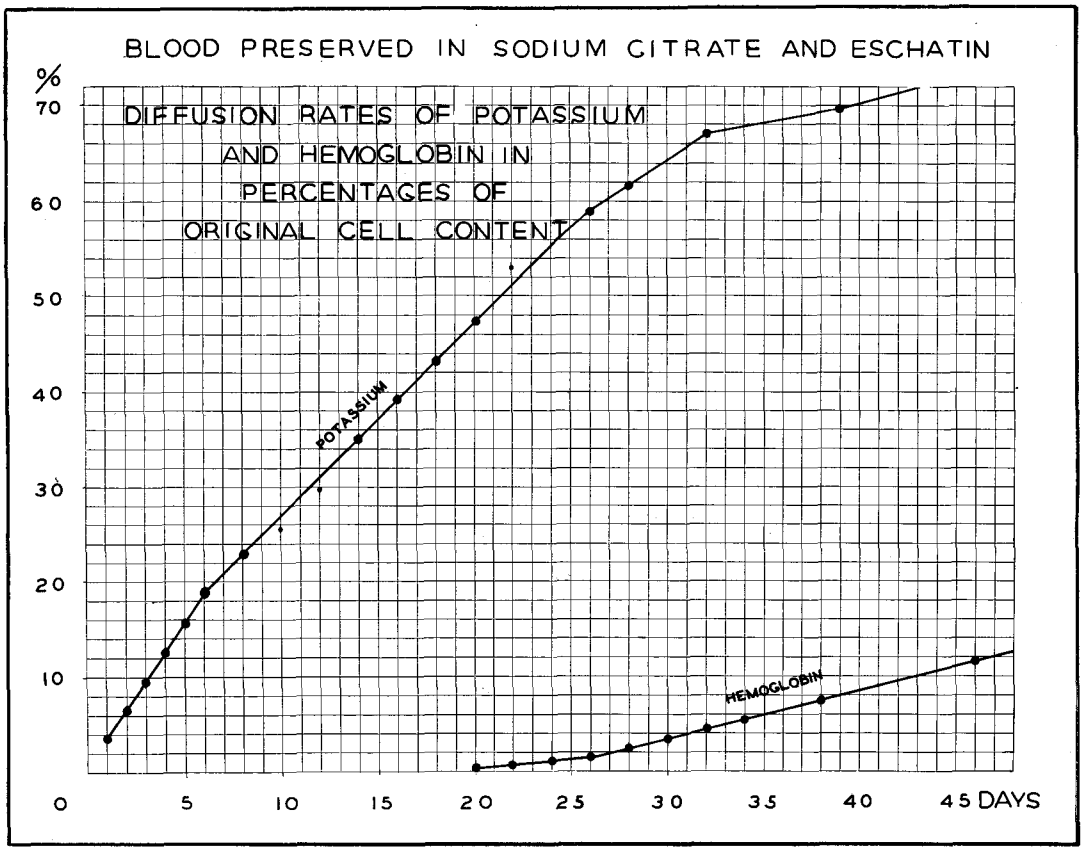
The blood of the professional donor, J. F., was of group O. To 125 cc. of blood was added 15 cc. of a 3 per cent sodium citrate solution and 10 cc. of eschatin (cortico

Table 7
Experiment 11
Blood Preserved in Diluted Russian Citrate Compound

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Milligrams Hemoglobin in Plasma Per 100 Cc. Blood	Culture (Blood Agar)
		Observed Value Per 100 Cc. Plasma	Sum of Increments Per 100 Cc. Blood	Sum of Increments From 100 Cc. Cells			
9/23/38	1	19.2	9.7	21.2	5.3	-	0
9/24/38	2	26.3	20.5	44.9	11.2	-	-
9/25/38	3	27.8	22.8	50.0	12.5	-	-
9/26/38	4	37.3	37.0	81.0	20.3	-	-
9/27/38	5	40.1	41.4	90.0	22.5	-	0
9/28/38	6	47.7	52.3	114.7	28.7	-	-
9/30/38	8	50.3	56.1	123.0	30.7	-	-
10/ 2/38	10	53.6	60.9	133.5	33.4	-	-
10/ 4/38	12	60.9	71.4	156.5	39.1	-	-
10/ 6/38	14	67.5	80.7	177.0	44.3	-	-
10/ 8/38	16	74.1	90.0	197.4	49.3	-	0
10/10/38	18	80.1	98.4	215.8	54.0	-	-
10/12/38	20	81.9	100.8	221.0	55.3	0.7	-
10/14/38	22	84.0	103.8	227.5	56.9	193.0	-
10/16/38	24	92.7	115.7	253.7	63.4	341.0	-
10/18/38	26	100.3	126.0	276.0	69.0	533.0	0
10/20/38	28	106.5	134.3	294.6	73.6	692.0	-
10/22/38	30	107.3	135.4	296.8	74.2	813.0	-
10/24/38	32	109.0	137.7	302.0	75.5	1172.0	-
10/31/38	39	111.0	140.2	307.5	76.9	1708.0	-
11/ 7/38	46	123.0	155.5	342.0	85.4	2242.0	-
11/14/38	53	128.3	162.3	356.0	89.0	3157.0	0

11/18/38 pH = 7.31 ± 0.05 as determined by glass electrode.

FIGURE 6



Data from experiment 12, table 8

Note that nearly 50 per cent of the cell potassium had diffused out before there was any evidence of hemolysis.

adrenal hormone); the mixture being treated as that of experiment 11.

Basic Values:

Same as in experiment 11 except that the pH of the citrate-eschatin mixture was 7.3.

These results are tabulated in table 8 and graphically represented in figure 6.

Experiment 13

Blood Preserved in Undiluted Russian Citrate Compound

The blood of the professional donor, J. F., was of group O. The procedure was similar to that of experiment 11 except that only one half the quantity of preservative was used and no distilled water was added. The 12.5 cc. of preservative contained 312.5 mg. of sodium citrate, to make a 0.228 Gm. per cent mixture.

The results are tabulated in teble 9.

Experiment 14

Blood Preserved in Grey's Buffered Sodution

The blood of the professional donor, J. F. was of group O. The procedure was the same as that in experiment 11 except that 112.5 cc. of blood was used and 12.5 cc. of preservative prepared according to Grey (150).

The results are tabulated in table 10.

Table 8
Experiment 12
Blood Preserved in Solution of Sodium Citrate and Adrenal Cortex Extract

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Milligrams Hemoglobin in Plasma Per 100 Cc. Blood	Culture (Blood Agar)
		Observed Value Per 100 Cc. Plasma	Sum of Increments Per 100 Cc. Blood	Sum of Increments From 100 Cc. Cells			
9/23/38	1	21.8	6.6	14.5	3.6	-	0
9/24/38	2	28.9	11.8	26.0	6.5	-	-
9/25/38	3	35.6	16.7	36.6	9.1	-	-
9/26/38	4	43.7	22.5	49.4	12.4	-	-
9/27/38	5	53.1	29.0	64.0	16.0	-	0
9/28/38	6	62.0	35.2	77.2	19.3	-	-
9/29/38	7	64.9	37.2	81.5	20.4	-	-
9/30/38	8	74.0	43.3	95.0	23.8	-	-
10/ 2/38	10	79.2	46.6	102.2	25.6	-	-
10/ 4/38	12	90.5	54.3	119.0	29.8	-	-
10/ 6/38	14	103.0	62.2	136.5	34.1	-	-
10/ 8/38	16	119.0	72.2	158.4	39.6	-	0
10/10/38	18	130.0	79.1	173.4	43.4	-	-
10/12/38	20	142.5	86.7	190.2	47.6	78.0	-
10/14/38	22	167.8	101.9	223.3	55.8	96.0	-
10/18/38	26	178.0	107.8	236.3	59.1	354.0	0
10/20/38	28	185.5	112.0	245.6	61.4	369.0	-
10/22/38	30	188.5	113.6	249.0	62.3	488.0	-
10/24/38	32	207.0	123.5	271.0	67.6	715.0	-
10/31/38	39	214.0	127.2	279.5	69.8	1203.0	-
11/ 7/38	46	231.0	135.5	297.0	73.3	1648.0	-
11/14/38	53	217.5	---	---	--	2180.0	0

11/18/38 pH = 7.51 $\pm$ 0.05 as determined by glass electrode.

Table 9
Experiment 13
Blood Preserved in Undiluted Russian Citrate Compound

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Milligrams Hemoglobin in Plasma Per 100 Cc. Blood	Culture (Blood Agar)
		Observed Value Per 100 Cc. Plasma	Sum of increments Per 100 Cc. Blood	Sum of increments From 100 Cc. Cells			
9/23/38	1	35.9	8.3	18.1	4.5	-	0
9/24/38	2	43.1	13.4	29.2	7.3	-	-
9/25/38	3	60.2	24.4	53.4	13.3	-	-
9/26/38	4	72.3	30.8	67.4	16.8	-	-
9/27/38	5	84.9	39.2	85.6	21.4	-	0
9/28/38	6	95.0	44.5	97.3	24.3	-	-
9/29/38	7	101.0	48.0	105.0	26.2	-	-
9/30/38	8	106.0	51.0	111.2	27.8	-	-
10/ 2/38	10	114.0	56.5	123.4	30.8	-	-
10/ 4/38	12	130.5	60.8	133.0	33.2	-	-
10/ 6/38	14	140.0	69.8	152.8	38.2	-	-
10/ 8/38	16	154.5	77.5	169.6	42.4	-	0
10/10/38	18	169.5	85.4	186.5	46.6	-	-
10/12/38	20	171.5	86.1	189.0	47.2	115.0	-
10/14/38	22	187.8	95.0	207.5	51.8	170.0	-
10/16/38	24	--	--	--	--	253.0	-
10/18/38	26	203.5	102.0	223.0	55.7	354.0	0
10/20/38	28	207.8	104.0	227.5	56.9	476.0	-
10/24/38	32	216.5	108.5	237.0	59.2	992.0	-
10/31/38	39	228.0	112.0	245.0	61.3	1492.0	-
11/ 7/38	46	232.5	114.2	250.0	62.5	2108.0	-
11/14/38	53	222.0	--	--	--	3554.0	0

11/18/38 pH = 7.45 $\pm$ 0.05 as determined by glass electrode.

Table 10
Experiment 14
Blood Preserved in Grey's Buffered Solution

Date	Days	Milligrams of Potassium			Percentage of Cell K Diffused Out	Milligrams Hemoglobin in Plasma Per 100 Cc. Blood	Culture (Blood Agar)
		Observed Value Per 100 Cc. Plasma	Sum of Increments Per 100 Cc. Blood	Sum of Increments From 100 Cc. Cells			
9/23/38	1	27.5	7.4	16.2	4.1	-	0
9/24/38	2	37.3	15.0	32.9	8.2	-	-
9/25/38	3	51.2	24.1	53.0	13.2	-	-
9/26/38	4	63.0	30.9	67.8	17.0	-	-
9/27/38	5	80.8	42.1	92.4	23.1	-	0
9/28/38	6	88.7	46.9	102.9	25.7	-	-
9/29/38	7	95.7	51.2	112.3	28.1	-	-
9/30/38	8	102.5	55.6	121.8	30.5	-	-
10/ 2/38	10	104.0	56.1	123.0	30.8	-	-
10/ 4/38	12	123.5	69.5	152.4	38.1	-	-
10/ 6/38	14	137.0	77.2	169.2	42.3	-	-
10/ 8/38	16	145.0	81.7	179.1	44.8	-	0
10/10/38	18	154.5	86.8	190.4	47.6	-	-
10/12/38	20	166.0	93.1	204.1	51.0	25.0	-
10/14/38	22	181.8	101.4	222.4	55.6	85.0	-
10/16/38	24	192.5	107.0	234.7	58.7	117.0	-
10/20/38	28	203.0	112.2	246.0	61.5	242.0	0
10/24/38	32	221.0	120.0	263.0	65.8	441.0	-
10/31/38	39	232.0	125.0	274.0	69.0	1049.0	-
11/ 7/38	46	247.0	131.1	288.0	72.0	1445.0	-
11/14/38	53	228.5	--	--	--	1658.0	0

11/18/38 pH = 7.50 ± 0.05 as determined by glass electrode.

Table 11
Experiments 11-14
The Difference in Rates of Hemolysis

Date (samples collected 9/22/38)	Days	Diluted Russian Citrate Compound		Sodium Citrate and Adrenal Cortex Extract		Undiluted Russian Citrate Compound		Grey's Buffered Solution	
		Hemoglobin in Plasma		Hemoglobin in Plasma		Hemoglobin in Plasma		Hemoglobin in Plasma	
		Grams	Per Cent	Grams	Per Cent	Grams	Per Cent	Grams	Per Cent
10/12/38	20	0.001	0.005	0.078	0.52	0.115	0.76	0.025	0.17
10/14/38	22	0.175	1.17	0.094	0.63	0.164	1.09	0.078	0.52
10/16/38	24	0.344	2.29	0.190	1.27	0.259	1.73	0.116	0.77
10/18/38	26	0.524	3.49	0.254	1.69	0.354	2.36	0.164	1.09
10/20/38	28	0.684	4.56	0.376	2.51	0.477	3.18	0.238	1.59
10/22/38	30	0.812	5.41	0.502	3.35	0.651	4.34	0.342	2.28
10/24/38	32	1.150	7.67	0.716	4.77	0.981	6.54	0.436	2.91
10/26/38	34	1.319	8.79	0.802	5.35	1.133	7.55	0.598	3.99
10/28/38	36	1.488	9.92	0.888	5.92	1.285	8.57	0.760	5.07
10/30/38	38	1.697	11.31	1.223	8.15	1.493	9.95	1.061	7.07
11/7/38	46	2.236	14.91	1.674	11.60	2.105	14.03	1.463	9.75
11/14/38	53	3.135	20.90	2.151	14.34	3.526	23.50	1.673	11.15

Table 12
Percentage Loss of Potassium from Cells
Comparison of Different Preservatives

Days	2.5 % Solution of Sodium Citrate	3 % Solution of Sodium Citrate	Russian Citrate Compound	Peyton Rous Compound	Diluted Russian Citrate Compound	Solution of Sodium Citrate and Adrenal Cortex Extract	Undiluted Russian Citrate Compound	Grey's Buffered Solution
7	18.7	18.8	23.1	19.0	30.5	20.4	26.2	28.1
14	32.8	28.7	40.9	37.2	44.3	34.1	38.2	42.3
21	44.3	40.8	60.0	44.2	56.1	51.7	49.5	53.3
30	48.7	44.7	66.0	53.4	74.2	62.3	58.1	61.2

Table 11 shows at a glance the difference in rates of hemolysis in the four types of preservatives. The increases of each are expressed in grams in the plasma of 100 cc. of blood and then as percentage of hemoglobin lost from the cells.

Table 12 expresses the percentage loss of potassium from cells.

The information gained shows that none of the preservatives prevented increase in plasma potassium, and of the preservatives analyzed sodium citrate functioned best. The blood kept under oil manifested the same changes. The reason underlying the mechanism of potassium loss is obscure.

Amberson (5) has shown that hemoglobin itself is not toxic to the vertebrate body if it has been freed from stromata; and, if the solution is properly balanced, infusions containing twelve to fourteen per cent may cause no abnormal reaction. The quantities present in the plasma of the most hemolyzed bloods under our observation have not approached this figure.

Summary Par

1. The erythrocytes of preserved blood lose potassium at different rates depending in part on the type of preservative. This loss begins before the diffusion of hemoglobin, Hence, the degree of hemolysis can not be used as an index of potassium loss.

2. A high degree of potassium diffusion may be present in the complete absence of hemolysis.

CHAPTER IV

Part III

The Toxicity of Potassium

Many stories of poisoning following accidental ingestion of potassium salts are retold in an interesting blok by Orfila, published in 1818, but the first experimental attempt to determine the mode of its action was reported by James Blake in Edinburgh in 1840. He observed that intravenous injections in dogs were followed by cardiac arrest and death. Six years later Bouchardat and Stuart-Cooper in a series of fifty experiments established the lethal doses for fish, frogs, fowl, dogs, and rabbits. Although this work was done in 1846, the conclusions are valid today, namely that the toxic action of potassium depends on the mode of administration, the rate of injection, the amount of potassium in the salt used, and the individual resistance of the animal.

Since that time many studies have been made. Effects on cold blooded animals were reported by Binet (1892) and Botazzi (1896). On warm blooded animals there has been an almost unbroken stream of investigation. Some of the more interesting and important observations have been made by the following authors:

1857	Claude Bernard	1904	Braun
1864	Grandeau	1906	Bouchard
1865	Guttmann	1907	Gautrelet
1865	Podcopaew	1909	Joseph and Meltzer
1870	Falck	1911	Mathison
1871	Bunge	1921	Kramer and Tisdall
1874	Aubert and Lehn	1934	Maranon
1878	Böhm	1936	Truszkowski and Zwemer
1881	Feltz and Ritter	1937	Zwemer and Scudder
1883	Bochefontaine	1938	Scudder and Zwemer
1885	Richet	1938	Marenzi
1892	Doriel	1938	Katz and Lindner
1898	Beck	1938	Scudder, Zwemer, and Whipple
1899	Gottlieb	1939	Gerschman
1900	Herringham	1940	Scudder

For a more complete summary of the older work the monograph of Webster and Brennan (1927) is recommended. For the later work, the thesis of Gerschman (417) is very complete.

Reported lethal doses, within a rather wide range, are fairly consistent for the various experimental animals when the procedures have been comparable. A few are listed in tables 13 and 14.

This poisoning is not a phenomenon peculiar to the animal kingdom alone. Osterhout (305) has demonstrated that alterations in the concentration of potash in certain plant cells or their natural fluid habitat profoundly effect many of their normal reactions.

Concerning the toxicologic effects of potassium there are many contradictory data, owing in part to the choice of the animal. There is, however, fairly uniform agreement that small doses of potassium increase, while large doses weaken and paralyze, the normal functions of the nervous,

Table 13
Lethal Doses of Potassium
(Gustav Bunge, 1871)

Animal	Weight, Kg.	Dose, Gm.	Salt	Time of Death	Authority
		1. Introduced into Stomach			
Rabbits	---	3	KCl	30 min.	Guttmann
		1.6-4	KCl	40-70 min.	Bunge
Dogs	6	16-20	KCl	1 hr.	Podkopaew
		48	KNO_3	1 1/2 hrs.	Orfila
		2. Subcutaneous Injection			
Rabbits	---	1-1.5	(KCO_3 (KCl (KNO_3	15-20 min.	Guttmann
	1.2	4	(KCl (KNO_3	47-335 min.	Falck
	1.2	3	KCl	1 1/2 hrs.	Bunge
Cats	---	8	KCl	1 1/4 hrs.	Bunge
		3. Intravenous Injection			
Rabbits	---	0.23	KCl	Immediate	Grandeau
Dogs	---	0.3	KNO_3	Immediate	Traube
		1-1.5	KCl	Immediate	Grandeau
		0.1	KCl	Immediate	Bunge
		0.6-1	KCl	---	Podkopaew
		4. Intra-arterial Injection			
Dogs	---	1.5	KCl	---	Podkopaew

Table 14
Lethal Intrevenous Doses of Potassium

(Since Bunge)

Year	Authority	Animal	Salt	Milligrams of Potassium per Kilogram
1881	Feltz and Ritter	Dog	KCl	48.00
1883	Bochefontaine	Dog	KCl	100.00
1892	Dogiel	Dog	KNO_3	20.00
1906	Bouchard and Oliver	Dog	KCl	39.90
1910	Joseph and Meltzer	Dog	KCl	38.30
1938	Marenzi	Dog	KCl	20.00

glandular, and muscular systems.

Most authorities attribute death to cardiac paralysis; among them are: Claude Bernard (29), Traube (386), Grandeau (149), Aubert and Dehn (12), Böhm (46), Dogiel (103), Feltz and Ritter (123), Bochefontaine (44), Binet (35), Hald (161), Mathison (280), Howell (186), Gross (153), and Wiggers (406).

Electrocardiograms taken in the course of conditions associated with hyperpotassemia (357), or after ingestion (358), injection (73), perfusion (137), or topical application (406) of potassium salts show a variety of changes. These range from slowing of the rhythm, decrease in PR interval and low voltage, to bundle branch block, ventricular fibrillation and cardiac arrest.

The respiratory and cardiac centers are other focal points of potassium action. Hooker (184) has demonstrated, by infusing the medulla of dogs, that an increasing concentration of potassium over calcium in the spinal fluid is followed by both respiratory and cardiac arrest.

Both Astolfoni (11) and Hald (161) noted constriction of vascular smooth muscle. Katz and Lindner (1938) made the important additional observation that potassium in small doses causes coronary dilatation; increasing amounts cause first dilatation and then constriction; and complete occlusion follows larger doses. This severe vasoconstriction was re-

lieved by the exhibition of sodium salts.

The effect of potassium on blood pressure according to McGuigan and Higgins (283) depends on the manner of injection and amount of the salt injected.

Kleeberg (225) reported a fall with intravenous administration, Aubert and Dehn concur with this opinion in general but reported a rise with very small intravenous doses (12).

Mathison states that intra-arterial medication usually causes a rise in blood pressure but both pressor and depressor effects are demonstrable (280).

Toxic oral doses are promptly vomited. Both Orfila (303) and Bunge (66) tell of early investigators who ligated the esophagus as soon as the salt had been swallowed in order to demonstrate that absorption from the gastro-intestinal tract may be toxic. Lethal doses, by mouth, are reported as being seventy to one hundred times that of intravenous ones.

Animal Experiments

To test again the toxic action of potassium, the rabbit and dog were selected for this study. The former has a high and the latter a low potassium content of the blood.

In our first set of experiments five rabbits were used. In three, the potassium chloride solution was injected rapidly into the jugular vein with immediate death after a

generalized convulsion. In two, the solution was injected into the peritoneum; one lived a week, the other recovered and was re-injected on the twelfth day intravenously. The summary is tabulated in table 15.

In the second group of experiments, four dogs were used in an attempt to determine more accurately what part the speed of injection plays in the production of toxic symptoms. A typical protocol is tabulated in table 16 and figure 7.

Interpretation of Protocol

The actual lethal dose was given when 275 cc. of fluid had run in. The additional 25 cc. was accidental and may account for the excessive rise in the last plasma potassium. The gradual fall in plasma specific gravity and plasma protein may be considered as an indication of blood dilution. The changes in the cell volume as recorded by hematocrit readings are more difficult to explain. The initial fall represents dilution, the secondary rise a sudden transport of fluid from the blood.

A summary of the dog experiments is presented in table 17.

Toxicity in Man

The literature is very meagre as regards the toxic action of potassium on man. Some early descriptions are found in Orfila's "Traité des Poisons" published in 1818.

Table 15
Rabbits Given Injections of Solution of Potassium Chloride

Rabbit	Weight, Kilogram	Concentration of Solution as Grams per 100 Cc.	Amount Injected as Cc.	Place and Speed of Injection	Potassium Chloride as Grams	Potassium as Milligrams per Kilogram	Results
1	2.01	0.381	550	Peritoneum Slowly	2.1	547	Died on seventh day
2	2.00	0.381	500	Peritoneum Slowly	1.96	514	Revived and given another injection on twelfth day
3	2.50	0.381	40	Jugular vein Rapidly	0.152	32	Convulsion; died at once
4	2.36	0.381	40	Jugular vein Rapidly	0.152	34	Convulsion; died at once
5	2.00	0.381	50	Jugular vein Rapidly	0.190	49	Convulsion; died in few minutes
2	2.00	1.16	42	Jugular vein Slowly	0.487	128	Convulsion; died in 15 min. 5 sec.

Table 16

Infusion into Dog of Isotonic (1.16 Gm. per Hundred Cubic Centimeters) Solution of Potassium Chloride

Time	Elapsed Time in Minutes	Infusion	Hematocrit Reading; Percentage of Cells	Specific Gravity of Plasma	Plasma Proteins Gm. per 100 Cc.	Whole Blood Potassium as Mg. per 100 Cc.	Plasma Potassium as Mg. per 100 Cc.	Source of Blood	Comment
10:25 a.m.	--	Before anesthesia	49.0	1.0262	6.56	24.5	22.7	Leg vein	Blood for base line slightly hemolyzed; given 30 mg. per Kg. of pentobarbital sodium
10:35 a.m.	0	After anesthesia; infusion started	44.6	1.0248	6.09	20.1	14.0	Leg vein	
10:44 a.m.	9	After 100 cc.	38.9	1.0240	5.81	24.2	20.2	Jugular vein	Rate of infusion 20 drops a minute after first 50 cc.

Table 16 (continued)

Infusion into Dog of Isotonic (1.16 Gm. per Hundred Cubic Centimeters) Solution of Potassium Chloride (continued)

Time	Elapsed Time in Minutes	Infusion	Hematocrit Reading; Percentage of Cells	Specific Gravity of Plasma	Plasma Proteins Gm. per 100 Cc.	Whole Blood Potassium as Mg. per 100 Cc.	Plasma Potassium as Mg. per 100 Cc.	Source of Blood	Comment
11:29 a.m.	54	After 200 cc.	40.2	1.0237	5.72	36.8	40.1	Jugular vein	Cyanotic, with irregular paired respirations, during a period when fluid went in too fast
11:39 a.m.	64	After 275 cc.	47.4	1.0231	5.52	49.2	62.1	Jugular vein	Convulsion, stopped breathing, sphincters relaxed; heart fibrillating
11:41 a.m.	66	After 300 cc.*	38.6	1.0195	4.28	67.0	172.0	Right side of heart	Heart stopped; blood clear; no hemolysis

At death: Potassium in cerebrospinal fluid from basal cistern, 51.8 mg. per hundred cubic centimeters and in urine, 74.4 mg. per hundred cubic centimeters

Autopsy: Heart dilated, no pericardial effusion, bladder full; no petechial hemorrhages

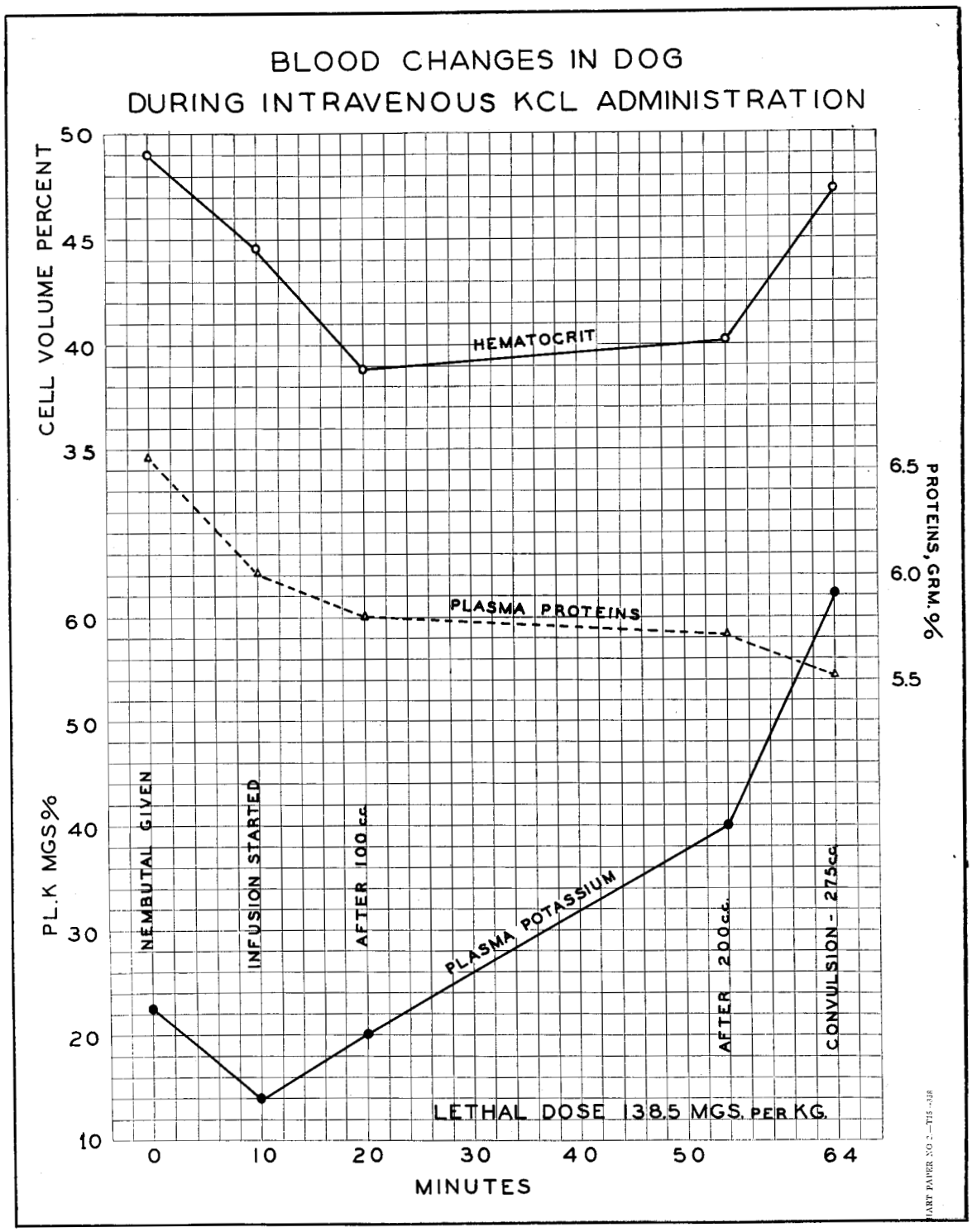
* 25 cc. accidentally run in rapidly after convulsion.

Table 17

Dogs Given Injections of Isotonic (1.16 Gm. per Hundred Cubic Centimeters) Solution of Potassium Chloride

Dog	Weight, Kg.	Solution Injected as Cc.	Potassium Chloride Injected as Gm.	Potassium as Mg. per Kg. of Body Weight	Time, Minutes	Plasma Potassium Before Injection, Mg. per 100 Cc.	Plasma Potassium at Death, Mg. per 100 Cc.	Comment
1	9.8	100	1.16	62.1	3 1/2	18.9	99.5	Pentobarbital sodium anesthesia, 30 mg. per Kg.; fluid run rapidly into vein by infusion; died after convulsion
2	10.3	120	1.392	73.0	18 1/2	22.8	51.5	No anesthesia; fluid administered by syringe, 10 cc at a time, slowly; died after a convulsion
3	8.8	100 some leakage into tissue	1.16	61.0	33	18.8	26.0	Pentobarbital sodium; infusion; young dog; died suddenly
						15.5	25.6	Spinal fluid
4	12.0	275	3.19	139.0	64	22.7	172.0	Pentobarbital sodium; fluid run in slowly by infusion; died after convulsion
							51.0	Spinal fluid

FIGURE 7



Data from table 16

Note gradual hemoconcentration with rise of plasma potassium level. Total dose 1.4 grams. Totally hemolyzed human blood contains 200 milligrams of potassium; 700 cc. would contain a dose similar to that which proved lethal in this dog in an hour.

He reports the following:

A man with periodic fever took 1 1/2 ounces (45 cc.) of potassium nitrate, thinking it was epsom salts, and died in ten hours.

A woman aged 40, suffering from hearburn, took 3 or 4 drachms (11 to 15 Gm) of potassium sulfide in 4 ounces (120 cc.) of water by mistake. Severe vomiting ensuded, followed by unconsciousness, the presence of black blood in the capillary system, especially of the lips and eyelids, and paralysis of the left side of the body. The action of the heart was barely perceptible and then failed. Autopsy showed the mouth and esophagus to be clean; the gastric mucosa was not greatly involved, except that here and there it was dry and red, with sulfur precipitates.

Many other cases are given, and in each where death was rapid the picture was one of shock, when delayed that of severe gastro-enteritis.

Bunge (1871) observed that small doses do not effect the pulse nor temperature.

Kylin, in 1925, injected 0.15 to 0.8 grams of potassium chloride intravenously and reported a fall of blood sugar.

Arden, in 1934, recorded a case in which 15 grams of a potassium salt were taken by mouth. In forty minutes symptoms of muscular weakness, parasthesia of hands and feet and a metallic taste in the mouth appeared and lasted three to four hours.

Electrocardiographic tracings following the ingestion of potassium salts equivalent to 4.3 grams of potassium have been reported (356).

Recoveries following toxic doses have been effected by artificial respiration, cardiac massage, oxygen, injections

of saline (4), and cortical extract (358).

Summary Part III

(1) The parenteral administration of potassium is associated with toxic manifestations of both muscular and nervous tissue together with a depression of the central nervous system.

(2) The almost specific action of potassium is on the heart and circulation, with disturbances varying from diminished cardiac output to immediate diastolic arrest.

(3) The lethal dose in animals (see tables) which we have reaffirmed is of the same magnitude as those previously reported.

(4) The rate of injection is of particular importance for when given slowly several times the usual lethal dose is tolerated.

CHAPTER IV

Part IV

The Fate of Cellular Elements and Prothrombin in Preserved Blood

In the preceding experiments a tenfold rise in plasma potassium was observed at the end of one month's storage. This was not due to bacterial contamination. It was increased by trauma, such as shaking, and was definitely modified by the area of interface area between the sedimented cells and the overlying plasma.

The question was raised as to whether this increase in plasma potassium was a pure diffusion process or whether it was due to actual cell destruction. At the time this investigation was started it was not known that similar investigation had been carried on in Russia and Spain (chapter III).

This aspect of the problem is reported here.

Experiment 1

Method

Five cubic centimeters of freely flowing venous blood were collected in each of 35 sterile, round-bottomed test tubes containing 5.0 mg. of heparin as an anticoagulant. Each tube was inverted three times, plugged with cotton,

and kept in a refrigerator at approximately 4° C. throughout the period of the experiment.

Each day one tube was taken from the refrigerator and centrifuged for one hour. After 0.5 cc. of plasma had been removed for potassium analysis and the blood had been thoroughly mixed by inverting the tube fifteen times, the following determinations were made:

Red cell count

Hemoglobin (Hellige)

White cell count

Differential white cell count

Platelet count

Mean cell diameter of red blood cells (Halometer method)

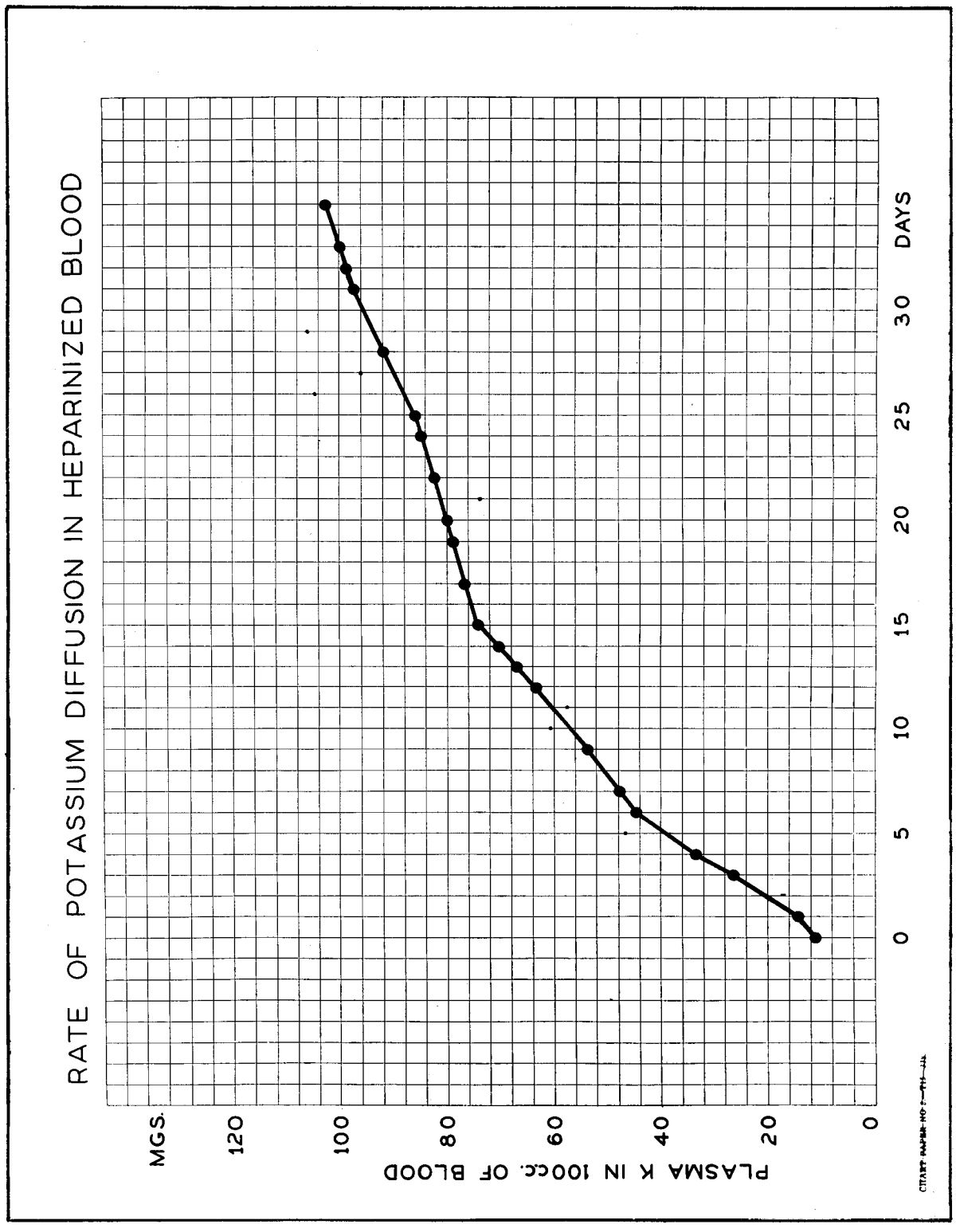
Plasma potassium

Results

The donor, A. J., was a professional of group O.

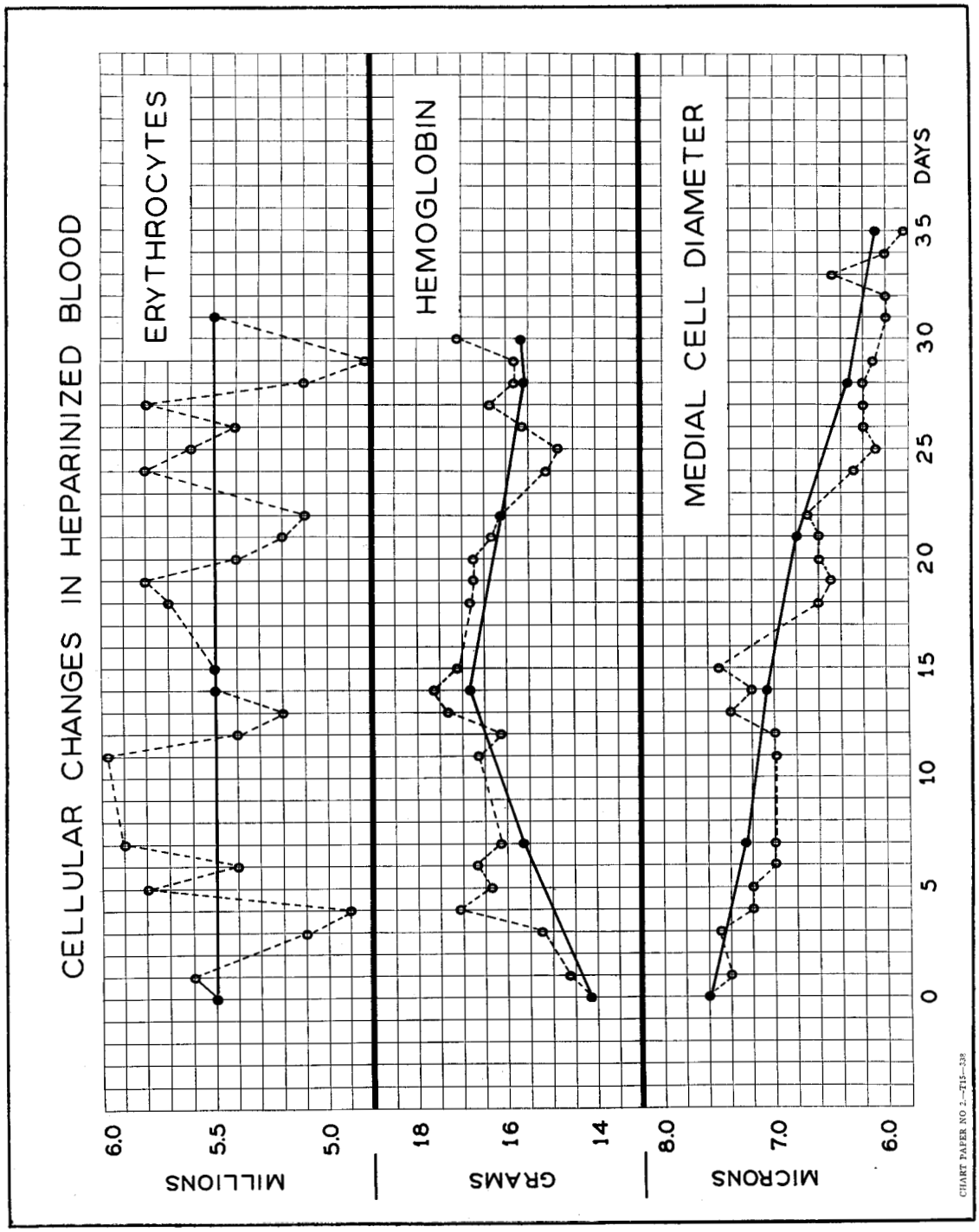
	Venous Blood		
	at start of Phlebotomy	at end of Phlebotomy (590 cc.)	
Hematocrit	46.0	44.7	per cent cells
Plasma specific gravity	1.0286	1.0266	
Plasma proteins	7.38	6.70	Gm. per cent
Whole blood potassium	212.0		mg. per cent
Plasma potassium	21.5		mg. per cent
Cell potassium (calculated)	434.0		mg. per cent
pH		7.51	

FIGURE 8



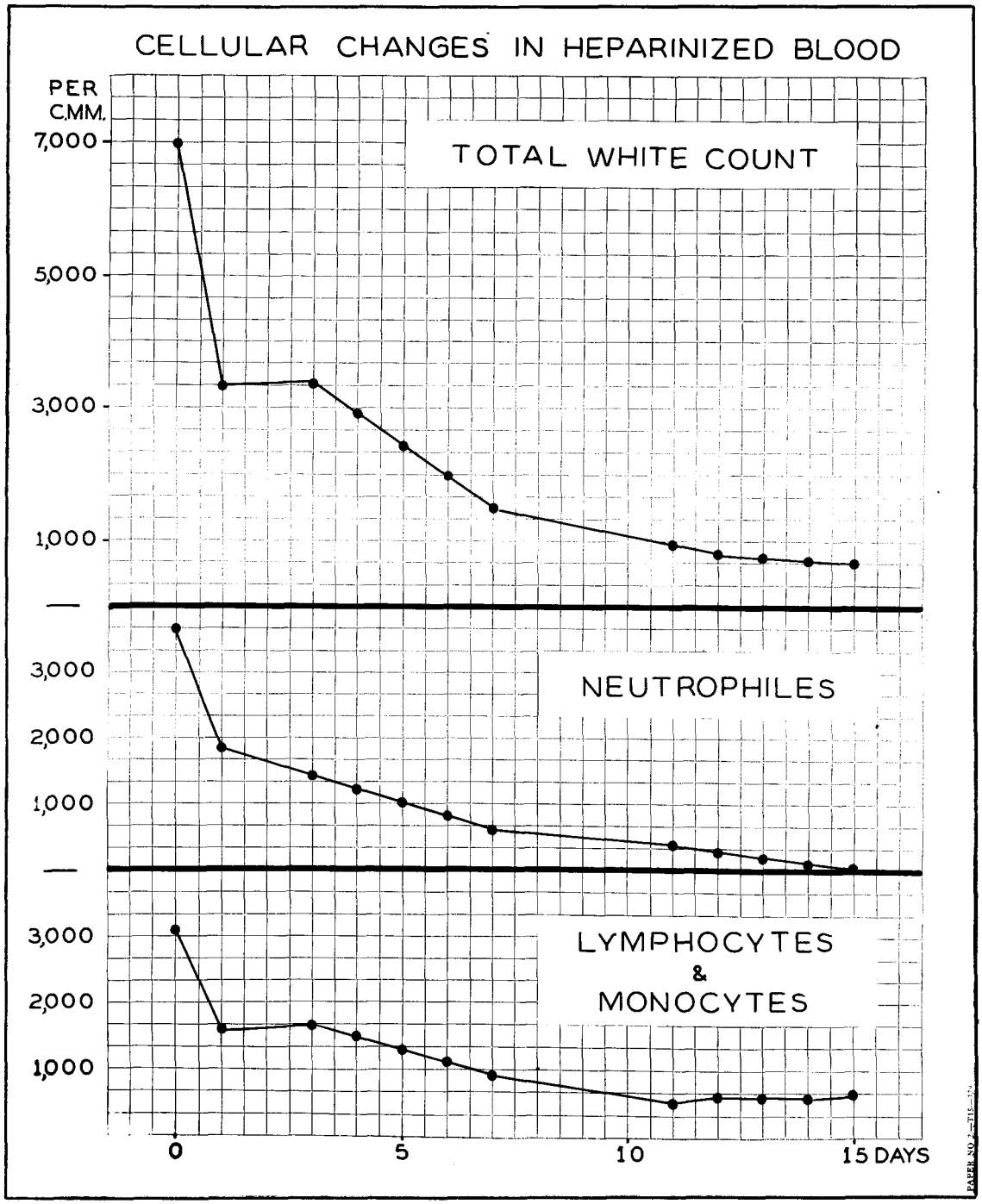
Each point on the chart represents a determination done on a different sample each day and represents the actual amount of potassium in the plasma of 100 cc. of blood.

FIGURE 9



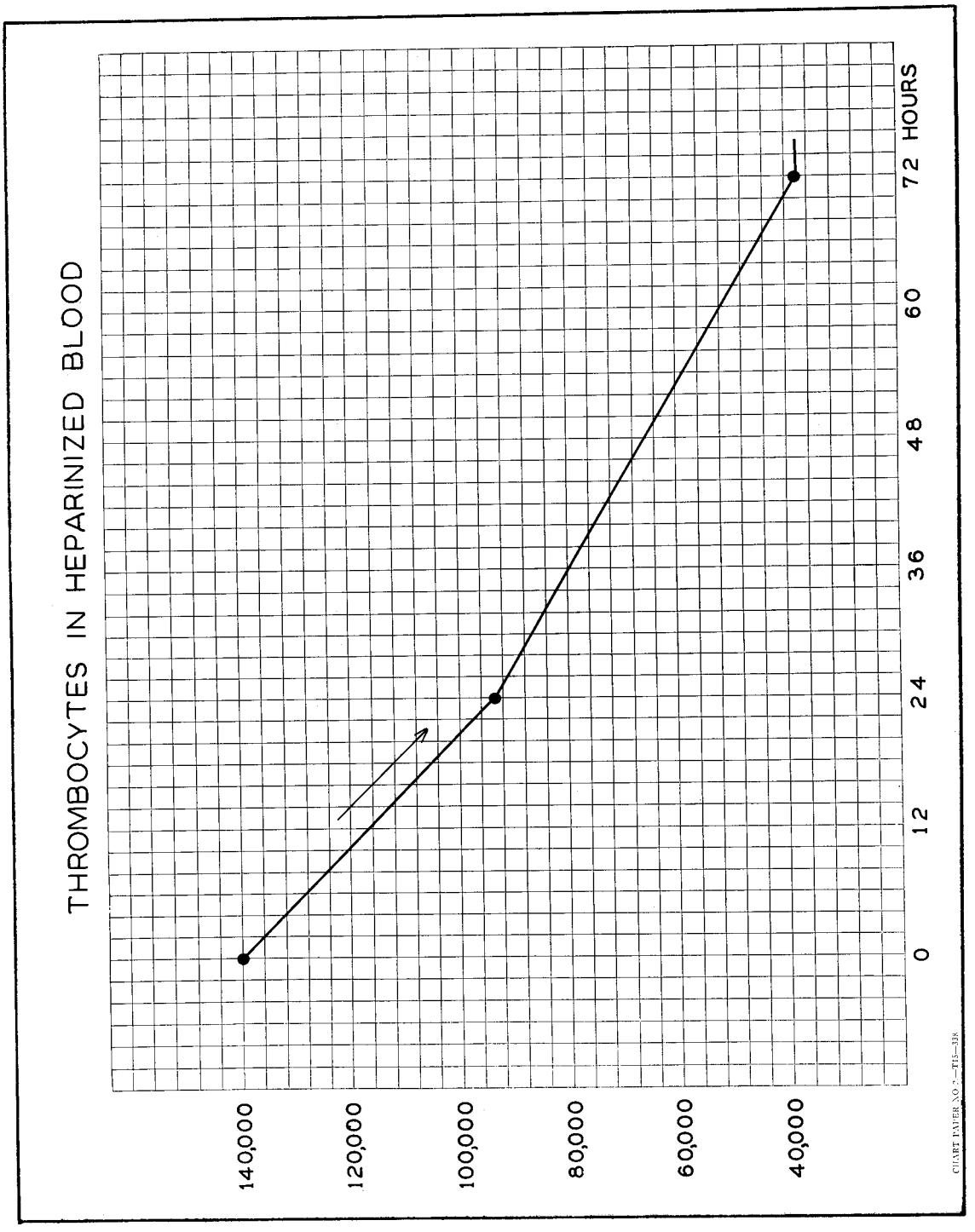
Erythrocytes varied between 4.8 and 6.2, the mean being 5.5 millions. Hemoglobin values varied between 14.2 and 17.6 grams per cent. The rise is due to evaporation; the fall prrobably due to hemoglobin removed in plasma for potassium determination. The size of the red blood cells gradually fell from 7.6 to 5.8 microns.

FIGURE 10



The total white cell count fell 50 per cent in 24 hours. They lost shape and disappeared by the sixteenth day. Range, 7000 to 675 cells. The neutrophiles showed most rapid changes. Their nuclei soon lost shape and disappeared. Range, 3600 to 14. Lymphocytes and monocytes disappeared more slowly. Basophilic and eosinophilic granules were well preserved for thirty days. They were few in number. Range, 3100 to 654.

FIGURE 11



Thrombocytes fell rapidly to about 40,000 and for thirty days remained approximately at this figure.

Values on Initial 5.0 cc. Sample*

Red blood cell count	5,500,000.0	
Hemoglobin	14.2	Gm. per cent
White blood count	7,000.0	
Differential white cell count		
Polymorphonuclear leukocytes	52.0	per cent
Eosinophilic leukocytes	2.0	per cent
Basophilic leukocytes	1.0	per cent
Lymphocytes	42.0	per cent
Monocytes	3.0	per cent
Platelets	140,000.0	
Mean cell diameter	7.4	
Plasma potassium	20.6	mg. per cent

The results are graphically represented in figures 8, 9, 10, and 11.

Experiment 2

Method

The procedure was exactly similar to that carried out in experiment 1 except that 0.5 cc. of 3.5 per cent sodium citrate was added to each 5.0 sample of blood instead of heparin.

Each day one tube was taken from the refrigerator and the following determinations were made:

Red cell count

Hemoglobin

White cell count

Differential white cell count

Platelet count

Fragility test

The plasma clotting time was done on two samples of

---

*Corrected for 0.5 cc. plasma removed.

50 cc. of blood by the method of Quick (330, 332) who postulates that the rate of coagulation is a function fo the concentration of prothrombin and that the production of thrombin in oxalated plasma is proportional to the concentration of prothrombin if an excess of thromboplastin is present and an optimal amount of calcium is added. The plasma was recalcified with calcium chloride at a constant temperature of 40° C. in the presence of human brain tissue emulsion to supply the thromboplastin substance, the end point being recorded by the shift on a photoelectric cell galvanometer. In determining the curve, a normal value of twenty seconds, as determined in this clinic by Doctor Kenneth Olsen, was used instead of the lower values suggested by Quick. (This standard was changed later.)

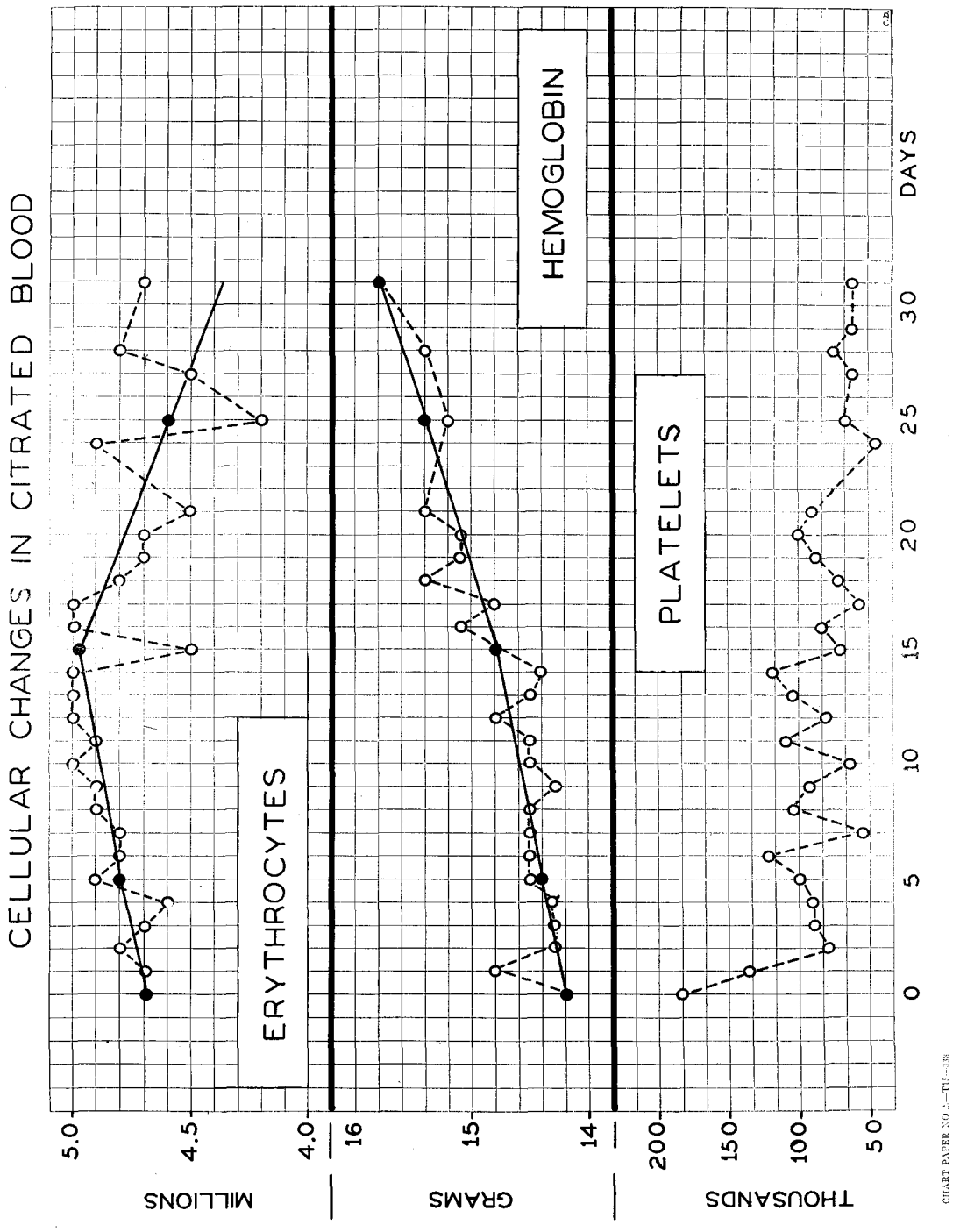
Results

The donor, W. T. S., was a volunteer.

Comparative Values of Blood in Heparin and in Sodium Citrate

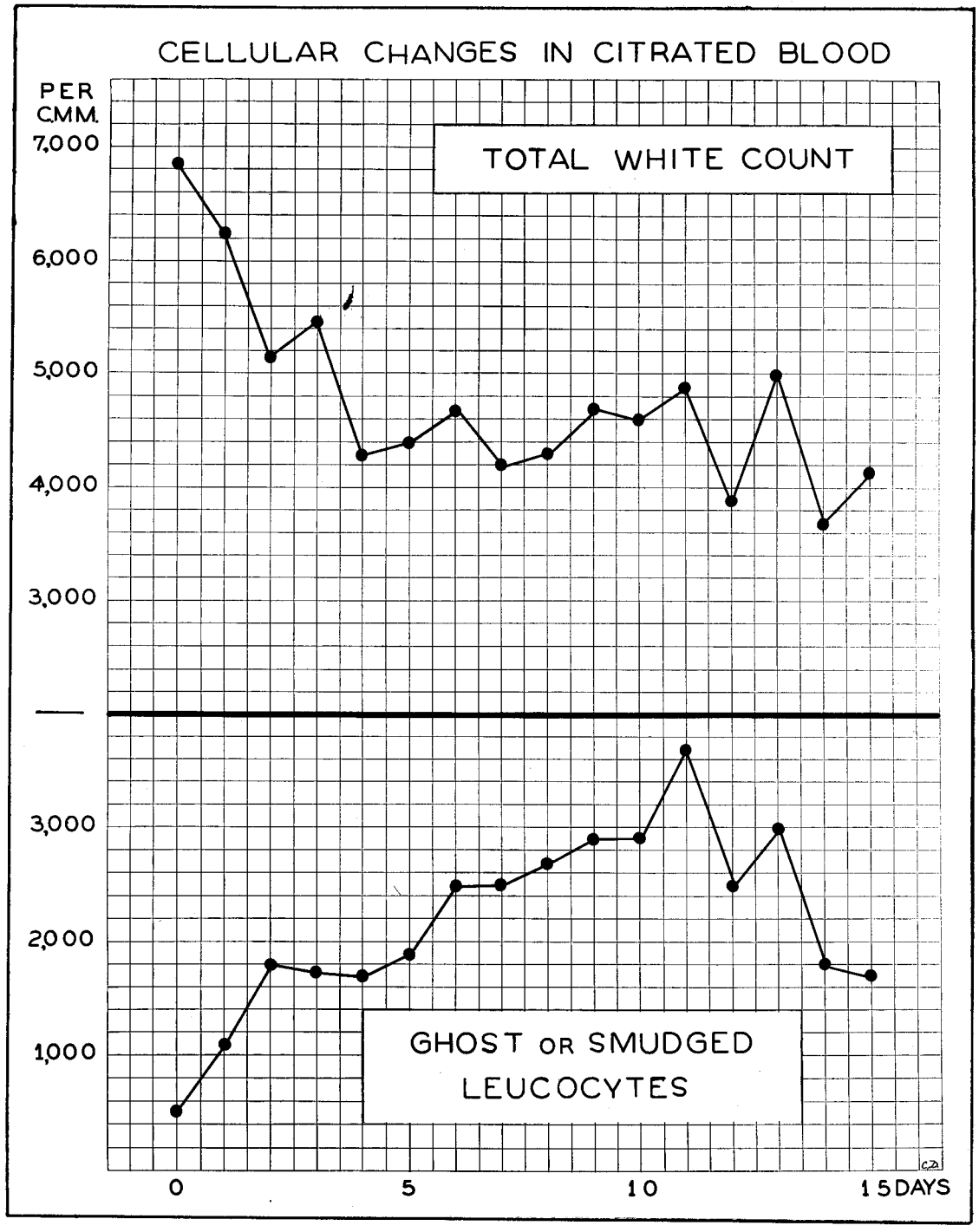
	Heparin	0.35 per cent Sodium Citrate
Hematocrit	45.5	42.3 per cent cells
Plasma specific gravity	1.0249	1.0238
Plasma proteins	6.12	5.75 Gm. per cent
Whole blood potassium	192.0	183.0 mg. per cent
Plasma potassium	17.5	17.2 mg. per cent
Cell potassium	377.0	409.0 mg. per cent
pH	7.49	

FIGURE 12



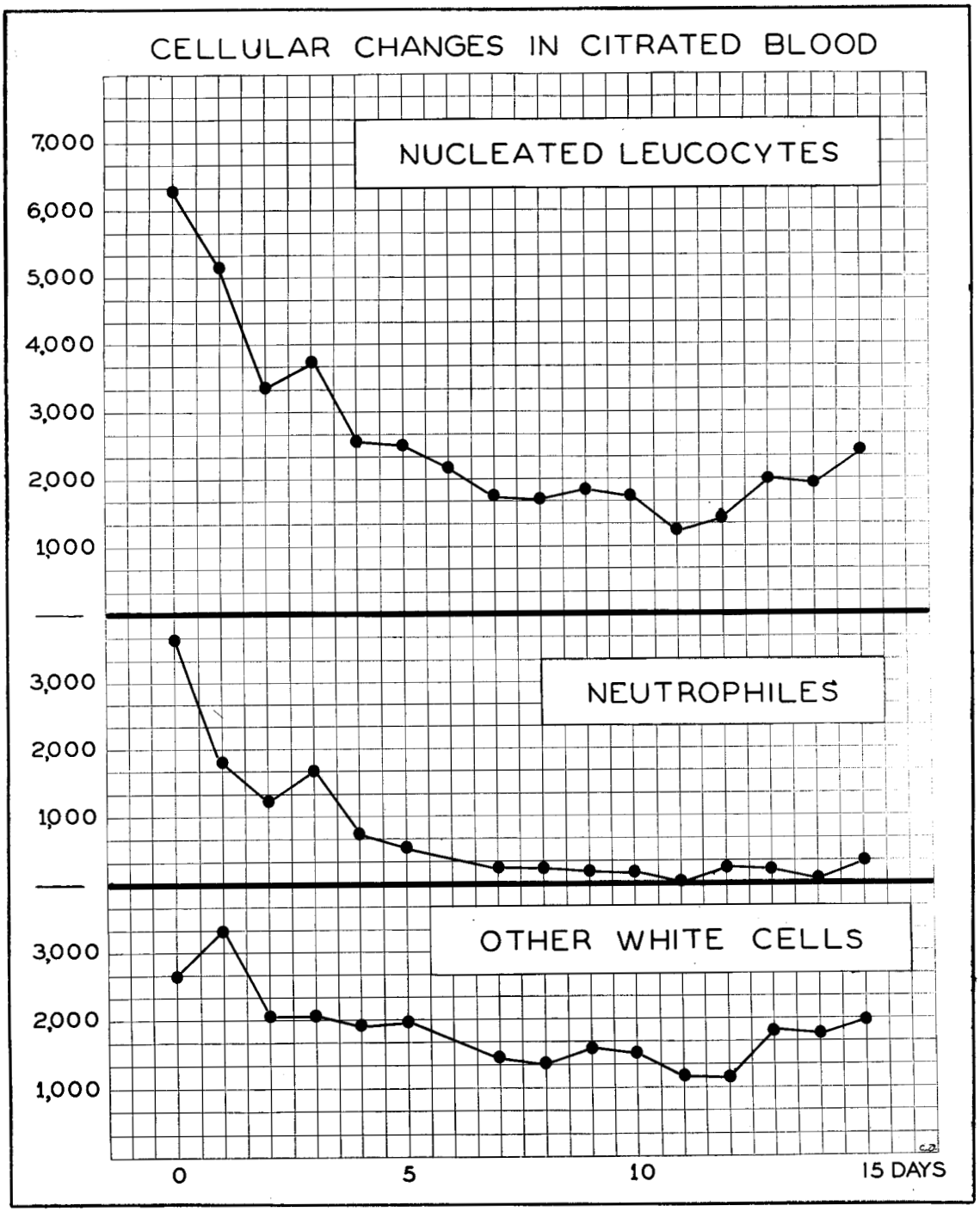
Red cell counts, corrected for dilution, varied between 5.5 and 4.6, the mean being 5.1 millions. Here there is an actual loss of 1,000,000 to 1,500,000 cells at the end of thirty days. Hemoglobin values varied between 15.6 and 16.3 grams per cent. The gradual rise is attributed to evaporation. Platelets fell from 206,800 to 87,800 in forty-eight hours, then remained constant for about fifteen days. After that counts were difficult.

FIGURE 13



The total white cell count as observed in the chamber fell 27 per cent in the first five days. Careful differentiation of cells in the chamber and observation on a slide showed that 75 per cent of the total had no nuclei or were so fragile that they broke and left only a smudge by the twelfth day.

FIGURE 14



The nucleated leucocytes, presumably the only ones capable of function, decreased nearly 50 per cent in the first three days. The polymorphoneuclear leucocytes diminished 50 per cent in 24 hours, accounting almost completely for the drop in total count. By the sixth day it was difficult to be sure that any remained. Eosinophiles were well preserved. Lymphocytes remained almost constant. Monocytes were difficult to differentiate.

Values on Initial Sample Corrected for Dilution in Citrate

Red blood cell	5,100,000.0	
Hemoglobin	15.6	Gm. per cent
White cell count	7,700.0	
Differential white cell count		
Polymorphonuclear leukocytes	61.0	per cent
Lymphocytes	29.0	per cent
Monocytes	7.0	per cent
Eosinophilic leukocytes	2.5	per cent
Basophilic leukocytes	0.5	per cent
Platelets	206,800.0	

The results of the counts and hemoglobin determinations are graphically represented in figures 12, 13, and 14.

(1) Fragility of Erythrocytes

The end points were poor throughout these studies. An actual curve of fragility could not be constructed on a daily basis. As late as the fifteenth day cells could be suspended in 0.45 per cent sodium chloride without complete hemolysis. Even on the thirtieth day cells carefully handled did not lake completely in 0.52 per cent sodium chloride.

Spontaneous hemolysis was first noted on the seventeenth day. Slight shaking in physiological saline after the tenth day caused hemolysis.

This is certain. The cells on the tenth day are slightly less resistant than those on the first, and those stored for thirty days are definitely more fragile than those stored for ten days.

(2) Prothrombin

In the first series, plasma clotting times were run for a period of fifty-two consecutive hours at hourly (toward the end, two hourly) intervals. This was done with the aid of Doctor Kenneth Olsen and Miss Hildegard Menzel. There was a rpid rise in clotting times in the first fifteen hours so that at the end of this period the prothrombin content had been reduced to ineffectual levels. The curve was quite similar to that later published by Rhoads and Panzer (334) and seemed to indicate that bloods stored for periods longer than a day would be ineffectual in treating hemorrhage which resulted from a deficiency of prothrombin.

An eight day old blood, however, was given a jaundiced patient with a hemorrhagic tendency, and bleeding stopped. This was reported to Doctor Olsen who made up a new extract of rabbit brain and repeated these tests on blood supplied to him from the blood bank. To his surprise, this time there was no sudden loss of prothrombin concentration. The figures for this experiment are not available at this time, Doctor Olsen being on leave of absence because of illness.

Through the courtesy of Doctor Grant Sanger, we have been able to obtain the following observations on separate bloods, each time being very careful to use fresh brain extract.

Discussion

Red Blood Cells

The maintenance of total erythrocyte counts at an

Table 18

Prothrombin Concentrations in the Plasma of Stored Blood

Date Blood Stored	Date Test Made	Age in Days	Prothrombin Concentration in Per Cent
10/27/39	2/21/40	117	47
10/27/39	2/21/40	117	38
11/20/39	2/21/40	94	41
12/15/39	2/21/40	69	49
1/31/40	2/21/40	21	52
2/28/40	3/12/40	13	100
3/ 1/40	3/12/40	11	100
2/16/40	2/26/40	10	68
2/23/40	3/ 1/40	8	84
2/23/40	3/ 1/40	8	84
2/26/40	3/ 5/40	8	60
3/ 4/40	3/12/40	8	100
3/ 4/40	3/12/40	8	80
3/ 4/40	3/12/40	8	100
2/19/40	2/26/40	7	100
2/19/40	2/26/40	7	76
2/23/40	2/29/40	6	100
2/23/40	2/29/40	6	100
2/16/40	2/21/40	5	74

approximately constant level in the heparinized blood and the moderate destruction of erythrocytes in citrated blood after the fifteenth day of storage suggests that cell destruction per se plays, at most, only a small part in the steady increase of the potassium content of the plasma and insures the recipient of receiving a large percentage of functioning cells after periods of storage of at least a month.

Hemoglobin

The hemoglobin content remains constant, though at the end of thirty days 20 per cent of it may be in the plasma.

Volume Index and Diameter of Red Blood Cells

The volume index decreases with age. The greater part of the decrease in volume seems due to the diminution in size of the red blood cells. At the end of thirty days, these cells may lose as much as 25 per cent of their chief base, 20 per cent of their hemoglobin, and as a result decrease about 20 per cent in diameter.

White Blood Cells

The polymorphonuclear leukocytes may show swelling, hazy cytoplasm, and poorly staining nuclear granules as early as twenty-four hours after storage. Disintegration

is extremely rapid and may play some part in the steep rise of the potassium curve in the first week; likewise it may play a part in decreasing bactericidal properties.

The lymphocytes are more resistant. At the end of thirty days they are easy to recognize when seen.

The eosinophiles are most resistant, the eosinophilic granules remaining particularly bright even when the nuclear material has faded or broken up.

Platelets

Platelets disintegrate more rapidly in heparinized blood than in citrated blood. In both they reach their low point in about three to five days. Those remaining stay fairly fixed until clumping makes counting difficult about the fifteenth day.

Prothrombin

The results using the Quick method of determining the prothrombin concentration yield values similar to those reported by Lord and Pastore (268) using the Brinkhous, Smith, and Warner method.

The results suggest that the use of brain extract which has been kept too long may account for the discrepancy between reported findings. To Doctor Olsen should go the credit for making this very essential observation.

Summary

(1) In heparinized preserved blood, there is little or no actual loss in the number of red blood cells over a period of thirty days; in citrated blood, there is some loss beginning about the fifteenth day and amounting to 1,000,000 to 1,500,000 cells by the end of the month.

(2) The hemoglobin content remains constant in the total sample though 15 to 25 per cent may diffuse out of the cells into the plasma in one month.

(3) The mean cell diameter of red blood cells in heparinized blood is reduced approximately 25 per cent in thirty days.

(4) The polymorphonuclear leukocytes are diminished by 50 per cent in forty-eight hours and are amorphous masses in fifteen days.

(5) The lymphocytes and eosinophiles do not disintegrate so rapidly; the latter are particularly well preserved. The monocytes are difficult to trace.

(6) The platelets fall rapidly in both heparinized and citrated blood, more so in the former than the latter, and remain at levels between 10,000 and 80,000 for about fifteen days.

(7) The fragility of red cells slowly increases with increasing age; exact curves are difficult to establish.

(8) The prothrombin level is maintained above 50 per cent of normal concentration for a period of at least four months. The use of old brain extract will cause clotting times which are too rapid, thereby giving a false picture of the true degree of efficacy of preserved blood in the therapy of hemorrhagic diseases associated with low prothrombin concentrations.

CHAPTER IV

Part V

Increasing Effect of Trauma with Advancing Age of Blood

In an earlier experiment (Part I) it was clearly shown that trauma to the blood in the form of shaking caused rapid laking of the blood and loss of increased amounts of potassium from the cells.

During the Winter of 1938-1939, studies were carried on at the Mt. Sinai Hospital Blood Bank through the courtesy of Doctor Nathan Rosenthal. It was noticed that at the end of the trip from Mt. Sinai to Presbyterian Hospital, a distance of about six miles, freshly drawn blood remained clear while older bloods transported at the same time became hemolyzed.

This raised a question, the answer to which would seem to be of practical importance. Is it preferable, for instance, in the case of war to draw blood from civilian populations back from the actual fighting districts and hold it in central depots at the point of withdrawal, or should it be shipped at once to the site of proposed use and then stored? An attempt to get this answer was made in the following manner.

Procedure

Ten cc. samples of blood were obtained from ninety-six healthy adult donors and transported at once from Mt. Sinai to Presbyterian Hospital.

At varying intervals the tubes were taken from the refrigerator. A sample of plasma was removed for potassium determination before the blood was disturbed, and then a second portion was taken after each tube had been inverted twenty times.

The results are recorded graphically in figure (15).

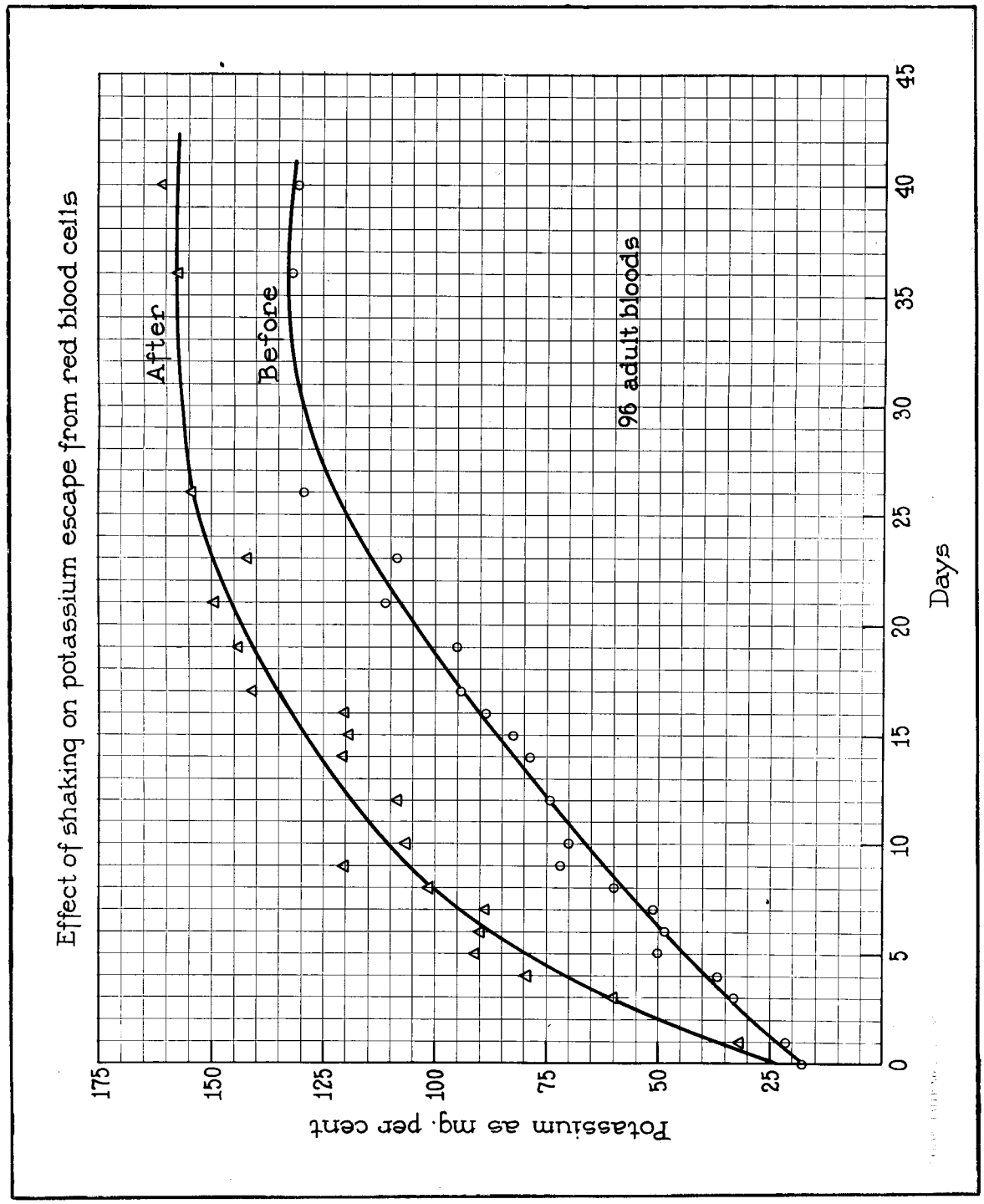
Interpretation

The curve representing the diffusion of potassium in the unshaken blood is of the same order as previously observed. Agitation caused an increase in plasma potassium in each case. During the first three days the increment was small; during the next three weeks it became gradually larger, and toward the end of the curve when potassium values approached equilibrium inside and outside the cells, the increment again decreased.

This would suggest that it would be more preferable to transport blood soon after withdrawal than to wait a week or more when less movement would cause more destruction of cells.

To check these findings a more controlled experiment was

FIGURE 15



In fresh blood shaking causes only slight increase, in older bloods a more marked increase, in still older bloods such a large percentage of potassium has already left the cells that the increase is less marked.

set up.

Method

The blood of two voluntary donors, C. R. D. and J. S., was drawn at weekly intervals and placed in 50 cc. colorimeter tubes containing 5 cc. of 3.5 per cent sodium citrate.

At the end of two weeks following the drawing of the last two samples, the previously drawn samples were removed from the refrigerator where they had been kept at a temperature of 5° and 6° C. since the time of the phlebotomy.

A small sample of the plasma was taken from each of the six tubes for potassium determination. Then all of the tubes were rotated end over end on a specially devised piece of apparatus for twenty minutes, centrifuged, and from each samples were taken for potassium determinations after the rotating.

Results

Since only comparisons between the levels before rotating and after rotating were sought, no attempt has been made to correct the figures for dilution caused by the anticoagulant, or in the figures obtained after rotating for the plasma removed to test the resting samples. After rotating values are, therefore, about twenty per cent too high but

since they are compared only to each other, the percentage change will indicate true differences.

Results are tabulated in table 19.

Discussion

This experiment was unsatisfactory in several respects. The results, however, are obvious. In one blood there was an increase of 8.5 per cent in the plasma potassium after rotating on the day it was taken compared with a 99.5 per cent increase at the end of two weeks. In the other blood the difference was greater 1.2 per cent when fresh to 132 per cent two weeks later.

Conclusion

The diffusion of potassium from red blood cells to the plasma following trauma increases rapidly with increasing age of the blood. This suggests that any transporting of blood should be done as soon as possible after its withdrawal from the donor to a place near the intended recipient.

This suggests that blood should be drawn and immediately transported to a place near the intended recipient.

Table 19
Increasing Effect of Trauma
with Increasing Age of Blood

Time in Weeks	C. R. D.			J. S.		
	Plasma Potassium as Milligrams Per Cent		Change in Per Cent	Plasma Potassium as Milligrams Per Cent		Change in Per Cent
	Before	After		Before	After	
0	14.1	15.3	8.5	20.8	21.3	1.2
1	45.8	74.0	~~61.5~~	52.1	121.0	132.1
2	60.1	118.0	99.5	60.3	140.0	132.2

CHAPTER IV

Part VI

Changes in Preserved Blood as a Function of the Composition and Shape of the Container

When Hertz (1893) attempted to use the recently introduced Hedin-Blix (169) hematocrit tube for estimating cell volume, he found his results much more uniform when the pipette had cod liver oil blown through it before use. Koeppe (229) used cedar oil and Freund (135) vaseline. Bordet and Gengou (1901) used paraffin to prevent coagulation during some of their now classical experiments in immunity and in 1911 Curtis and David applied this principle to the practice of transfusion, a method which reached its peak in the Kimpton-Brown paraffin tube method. "Athrombit" is the latest result of this effort to prevent coagulation (24). It has been pointed out that enough alkalie may be dissolved from ordinary glass (119) to cause toxic reactions in a patient and in the preceding experiments it has been shown that the shape of the flask undoubtedly plays a part in the rate of electrolyte distribution between the cells and plasma.

It was felt, therefore, that it would be of interest to determine by chemical studies, if possible, the effect of the material of which the container is made on the rate of

cell changes.

Procedure

A professional donor, International group O, was used to obtain a total of 590 cc. of blood, a part of which was used for this experiment. Phelbotomy was carried out with a number 15 Lewison needle, the systolic pressure being kept at a level of 100 mm. by the use of a sphymomanometer.

To establish a base line of values, a sample of blood was drawn into heparinized tubes at the beginning of the phlebotomy and another at the end.

The findings were as follows:

	Hematocrit %	Plasma Sp.Gr.	Plasma Proteins Gm. %	Potassium Mg. %		
				Plasma	Whole Blood	Cell
Initial	46.0	1.0286	7.38	21.5	212.0	435.0
Final	44.7	1.0266	6.70	21.7	192.0	403.0
Average	45.3	1.0276	7.04	21.6	202.0	419.0

To 350 cc. of blood was added 50 cc. of a 2.5 per cent solution of sodium citrate and the citrated blood distributed as follows:

(1) 25.0 cc. in each of four 50 cc. pyrex Erlenmeyer flasks.

(2) 25 cc. in each of four 50 cc. quartz flasks.

(3) 50 cc. in a 50 cc. plain glass colorimeter test tube.

(4) 50 cc. in a 50 cc. plain glass colorimeter tube whose inner surface had been completely covered by a thin layer of paraffin (melting point 56°-58° C)

(5) 50 cc. in a 100 pyrex Erlenmeyer flask.

(6) 50 cc. in a 100 cc. quartz flask.

Plasma potassium determinations were done as in previous experiments, each 25 cc. flask serving as a separate sample at weekly intervals and samples from the longer flasks being taken at the end of the second, third and fourth weeks.

The results are presented in table 20.

Discussion

The most obvious result is that the rate of breakdown in the long colorimeter tubes (3) and (4), either plain or paraffined, is roughly half as fast as that in the Erlenmeyer flasks of either pyrex or quartz. In the similar shaped flasks the quantity of blood did not play a large part, (1) and (5). It would seem that the adding of paraffin to the test tube did not diminish the rate of disintegration but rather increased it. When the rates in (1) and (2) are compared with those in (5) and (6), it becomes apparent that some factor other than the difference between quartz and pyrex must be

Table 20
Potassium Diffusion in Blood Stored
in Containers of Different Materials
and of Different Shapes

Container	Plasma Potassium at Weekly Intervals as Milligrams Per Cent				
	0	1	2	3	4
(1) 25 cc. Pyrex flask	21.6	81.6	115.6	137.0	192.0
(2) 25 cc. Quartz flask	21.6	79.6	86.7	119.0	145.0
(3) 50 cc. Glass tube	21.6	--	53.5	65.7	84.5
(4) 50 cc. Paraffined tube	21.6	--	52.8	69.3	94.5
(5) 100 cc. Pyrex flask	21.6	--	111.2	142.0	195.0
(6) 100 cc. Quartz flask	21.6	--	112.7	149.0	183.0

playing a part for the rate of change in the quartz flask in (2) is 25 per cent lower than the pyrex flask (1), while in (6) the rate is only 7 per cent less than in (5).

Knowing from previous observations that the area of interface might play a large role in these differences, these values were reduced to the common denominator of milligrams of potassium in the plasma of 100 cc. of blood at the end of 30 days and correlated with the interface diameters, areas, and square roots of the areas of the bloods in the various containers. These results are presented in table 21.

These values now show a direct relationship between the diameter of the container at the interface between the cells and plasma and the rate at which the cells lose potassium.

In this short series of observations, the values expressed as a function of the area are a little too small; as a function of the square root of the area, a little too large; the closest parallelism is between the diameters and the potassium levels. As an example in (5) and (6), the values actually are: 107.2 : 100.6 :: 6.6 : 6.1. If the relationship were a perfect one, it would be as follows: 107:2 : 98.9 :: 6.6 : 6.1. These values are well within the realm of experimental error in this particular series. To establish this relationship as a constant would necessitate,

Table 21

Potassium Diffusion Expressed as a Function of (1) The Diameter, (2) The Area, and (3) The Square Root of the Interface Between the Cells and Plasma in Different Types of Containers

Container	Plasma Potassium Per 100 Cc. of Blood	Interface Between Cells and Plasma		
		Diameter cm.	Area	Square Root of Area
(1) 25 cc. Pyrex flask	105.6	4.7	17.3	4.16
(2) 25 cc. Quartz flask	79.7	3.1	7.5	2.75
(3) 50 cc. Glass tube	46.5	2.2	3.8	1.95
(4) 50 cc. Paraffined tube	52.0	2.2	3.8	1.95
(5) 100 cc. Pyrex flask	107.2	6.6	34.2	5.85
(6) 100 cc. Quartz flask	100.6	6.1	29.1	5.40

of course, a sufficient number of estimations to treat the results satisfactorily.

In this imperfectly set up experiment, it is impossible to determine what changes, if any, are directly the result of the nature of the surface with which the cells come in contact. There is suggestive evidence that the lining of a glass tube with paraffin increases the rate of diffusion of potassium.

Summary

1. Differences in the rate of cell disintegration as measured by the rate of potassium diffusion could not be established as a function of the material in the walls of the container. There is some evidence to suggest that a paraffin coat increases rather than decreases the rate of cell breakdown.

2. The evidence suggests that with constant mass, the rate of diffusion varies directly with the diameters of the container at the interface between the cells and plasma.

CHAPTER IV

Part VII

Changes in Placental Blood

Following the announcement by Goodall, Anderson, Altimas and McPhail (1938) of the use of placental blood for both immediate use in obstetrics and as a source of supply for the other departments of their hospital, it was felt that the rate of disintegration as measured by potassium loss from the cells would be interesting to compare with those obtained from studies of adult blood.

Method

The first step consisted in collecting 5 cc. samples in heparinized hematocrit tubes for base line determinations as had been done in previous experiments. Typical results are shown in table 22.

This range of values has been confirmed by many subsequent studies. A longer series would not appreciably change the mean values.

When compared with mean values established for adult healthy donors, these facts establish themselves: (1) The cell volume as measured by the hematocrit is approximately 26 per cent higher than for an adult male, 34 per cent higher than for an adult female and 50 to 60 per cent higher than

Table 22
Normal Values for Placental Blood

Number	Cell Volume in Per Cent	Plasma Specific Gravity	Proteins Grams Per Cent	Milligrams Per Cent		
				Plasma Potassium	Whole Blood Potassium	Cell Potassium
1	53.9	1.0250	6.15	22.4	234	412
2	63.0	1.0261	6.53	21.4	279	430
3	66.1	1.0280	7.17	27.2	279	408
4	52.0	1.0250	6.15	26.4	225	407
5	50.1	1.0250	6.15	21.4	242	461
Average	57.0	1.0258	6.43	23.7	252	423

that of the average mother (145) at term.

The specific gravity of the plasma and plasma proteins are approximately 10 per cent lower than the adult; the plasma potassium, 40 per cent higher; whole blood potassium, 25 per cent higher, and cell potassium 5 per cent higher.

Subsequently larger quantities of blood were collected in different preservatives and tested at various fairly regular intervals. Six bloods were studied for periods of seventy days each: The results were so variable (ranging in plasma potassium content from 45 to 214 milligrams per cent at the end of forty days) that they could not be interpreted. Where the Montreal Clinic apparently had no trouble getting 75 to 300 cc. of blood, the residents of Sloan Hospital (through the courtesy and interest of Dr. Virgil C. Damon) had great difficulty in getting such quantities and in most instances when a sufficient quantity had been obtained for studies, the ratio of blood to anticoagulant was not known accurately enough to be sure of any results. This phase of the study needs to be completely reconsidered. In only one study, out of a series done for a period extending over a year were the results trustworthy. It is reported here.

Method

Two cylinders each capable of holding 42.8 cc. of fluid were used to collect blood from the umbilical cord of a full

term healthy primiparous mother. One contained 21.4 cc. of the Moscow I. H. T. solution which is made up of 0.7 per cent sodium chloride, 0.5 per cent sodium citrate, 0,02 per cent potassium chloride, and 0.0004 per cent magnesium sulfate. The other contained 4.2 cc. of a 3.5 per cent sodium citrate solution.

Results

Basic Values (Heparinized Blood)

Hematocrit	58.5 per cent cells 41.4 per cent plasma
Plasma specific gravity	1.0264
Plasma proteins	6.63 grams per cents
Plasma potassium	23.7 mg. per cent
Volume of cells in I.H.T.	12.54 cc.
Volume of fluid (plasma plus I. H. T.)	30.26 cc.
Volume of cells in citrate	22.6 cc.
Volume of fluid (plasma plus citrate)	20.2 cc.

Factor for correcting for dilution in I. H. T. solution, 1.38.
Factor for correcting for dilution in citrate solution, 0.42.

The results are shown in table 23.

Discussion

Here, as in the adult blood, the 0.31 per cent sodium citrate solution was superior to the more complicated I. H. T. solution. The rate of cell disintegration as measured by the rate of potassium diffusion was approximately 39 per cent slower

Table 23

Comparison of Rates of Cell Disintegration in Placental Blood Stored in I. H. T. Solution and in Sodium Citrate Solution

Date	Day	Milligrams of Potassium			
		I. H. T.		Sodium Citrate	
		Observed Value per 100 Cc. Plasma	In Plasma of 100 Cc. Blood	Observed Value per 100 Cc. Plasma	In Plasma of 100 Cc. Blood
8/17/39	0	23.7	9.8	23.7	9.8
8/19/39	2	24.2	33.4	55.0	32.5
8/26/39	9	49.9	68.9	133.0	55.9
9/ 2/39	16	64.2	88.6	136.0	57.1
9/ 9/39	23	77.9	107.5	184.0	77.2

in the former than in the latter.

The early values in placental blood are higher than those in citrated donor blood of the same strength, undoubtedly due to the greater handling at collection in the former. The rates in this particular experiment were slower after the first few days. This may have been due to the fact that the placental blood was stored in an elongated cylinder while the adult blood was stored in Erlenmeyer flasks. When the values are corrected for this difference in interface area, the values are strikingly similar (See Part I).

Conclusions

(1) The cell volume of placental blood is approximately 50% higher than that of the mother and 25% higher than that of a normal adult.

(2) There is a striking degree of similarity between the rates at which potassium leaves placental blood and adult blood when each is preserved in a 0.31 per cent sodium citrate solution.

(3) Using potassium diffusion rates as an index of cell disintegration, a 0.31 per cent sodium citrate solution is superior to the more complex Moscow I. H. T. solution as a preservative for placental blood.

CHAPTER IV

Part VIII

Plasma Potassium of Cardiac Blood at Death

In a preceding series of observations (Chapter II) the average plasma potassium level in dogs at death was found to be 15.2 M. eq./L. Winkler, Hoff and Smith (1938) reported similar levels, 14-16 M. eq./L and suggested that if the critical concentration for a man is comparable to that for the dog there is a wide margin of safety for the human being "since serum potassium would have to be increased by some 9 mM, per liter to reach a fatal level."

That there may be some difference in the sensitivity of the cells of different animal species is suggested by the observations of Scudder, Zwemer, Truszkowski and Whipple (357, 358), from this laboratory. They found the concentration of potassium in cardiac blood of cats at death varied between 9.5 - 11.4 M. eq./L (357).

Although there is only suggestive evidence that the mechanism of cardiac arrest is similar in the cats who died of shock produced by various methods, in the dogs who were poisoned with potassium chloride and in patients dying of a variety of diseases, it seemed essential to know what levels the potassium concentration in the cardiac blood of

Table 23A
Plasma Potassium of Cardiac Blood at Death

Animal	Number of Animals	Cause of Death	Range	Average	Average
			Mg.per Cent	Mg.per Cent	M.eq./L
*Cats	4	Intestinal obstruction	33.8-66.6	44.5	11.4
Cats	2	Intestinal fistula	40.6-45.5	43.1	11.0
Cats	4	Hemorrhage	25.0-57.4	46.5	11.9
Cats	4	Trauma	30.6-41.0	37.2	9.5
	14	Total		42.8	10.9
Dogs	4	Intravenous isotonic KCl	26.0-99.5	59.5	15.2

* These experiments were not done in the present series but have been added from [illegible] to make comparisons more complete.

humans reached at death.

To establish normal values the plasma potassium was determined on the venous blood of sixty healthy individuals who presented themselves as donors at the Mt. Sinai Hospital Blood Bank. This was made possible through the courtesy of Dr. Nathan Rosenthal.

The values are presented in the following table:

Table 24

Plasma Potassium of Normal Venous Blood*

	Mg. per Cent	M. eq./L.
Average (mean)	17.2	4.4
Median	17.2	4.4
Range	13.5-21.5	3.4 - 5.5
Standard Deviation	0.33	.08
Coefficient of Variation	1.9%	

* This group comprised 50 males and 10 females. Each value represents the mean of two aliquots of the original sample, 0.5 cc. of plasma being used.

Procedure

At the time of death, heart's blood of thirteen patients was withdrawn by cardiac puncture with a sterile needle and syringe. From 5 - 6 cc. of this sample were

introduced into a Sanford-Magath hematocrit tube containing heparin; gently mixed, capped, and centrifuged at 2,500 r. p. m. for an hour. The plasma was removed immediately from the cells and the potassium content determined.

The results are tabulated in table 25.

Comment

Cardiac arrest appears to be associated with different concentrations of potassium, not only for different species but also for different individuals within the species.

The narrow range of potassium between that found in normal circulating venous blood and in cardiac blood plasma at death indicates that human cardiac muscle and its associated nervous mechanism is probably more susceptible to variations in concentration than those of dogs and cats. The lethal levels, therefore, may not be comparable and the margin of safety not as great as animal experiments might indicate.

Summary

1. In cats dying from varied types of induced shock, the average concentration of plasma potassium in the heart's blood taken at the time of cardiac arrest was 42.8 mg. per cent (10.9 M.eq./L).

Table 25

Plasma Potassium of Cardiac Blood at Death

Date	Initials	Age, Sex	Hospital Number	Diagnosis	Operation	Plasma Potassium	
						Mg. per Cent	M.eq./L
8/29/37	W.N.	62 M	527186	Multiple fractures and contusions; laceration of aorta	Débridement	28.5	7.3
9/23/37	V.M.	9 M	530304	Multiple fractures. Hemoperitoneum	None	29.5	7.5
10/14/37	N.L.	28 F	530208	Idiopathic gastro-intestinal hemorrhage	Exploratory	24.0	6.1
11/23/37	F.F.	58 M	536668	Perforated duodenal ulcer	None	31.9	8.2
2/24/38	D.P.	25 F	542008	Paraganglioma of adrenal cortical tissue	Partial resection. Operative death	34.1	8.7
6/ 5/38	M.L	45 F	374569	Chronic cholecystitis, cholelithiasis, subphrenic and subhepatic abscesses	Cholecystectomy, incision and drainage abscesses	28.6	7.3
9/12/38	J.Mc.	70 M	556217	Carcinoma of colon with metastases to liver	Exploratory	26.3	6.7

Table 25

Plasma Potassium of Cardiac Blood at Death
(continued)

Date	Initials	Age, Sex	Hospital Number	Diagnosis	Operation	Plasma Potassium	
						Mg. per Cent	M.eq./L
9/27/38	M.K.	26 F	550662	Intestinal obstruction complicating pregnancy	Ileostomy	28.9	7.4
11/ 4/38	L.W.	31 F	549037	Mesenteric thrombosis	Enterectomy	38.0	9.7
11/ 7/38	E.Mc.	62 F	564918	Acute pancreatitis	None	31.6	8.1
12/ 13/38	L.V.	73 F	560067	Diabetic gangrene	Amputation	32.6	8.3
2/17/39	L.H.	58 F	566522	Carcinoma of breast	Mastectomy	26.1	6.7
2/24/39	A.M.	55 M	572368	Pneumonia, type III		27.9	7.1
Average						29.8	7.6

Standard deviation from the mean 3.6 mg. Coefficient of variation 14.5 per cent.

2. In dogs, following intravenous injections of isotonic potassium chloride in lethal doses, the concentration amounted to 59.5 mg. per cent (15.2 M.eq./L.)

3. In man the average at death was 29.8 mg. per cent (7.6 M.eq./L) compared to an average of 17.2 mg. per cent (4.4 m. eq./L) in the venous bloods of sixty health human adults.

CHAPTER IV

Part IX

Serum Potassium of Cadaver Blood

Cadavers were the first source of blood utilized on a large scale for storage and later use (Chapter I). Out of this practice grew the present wide spread use of living donors, as a source of supply for storage depots.

In preceding experiments it has been shown that living donors' cells gradually disintegrate as they are stored and any form of trauma will hasten this process of deterioration.

It seems logical, therefore, to suppose that cells already subjected to the injurious forces and changes incident to the death of a patient would have suffered some harm during the process, and would probably show more rapid changes when preserved after withdrawal.

To test this presumption cardiac bloods were obtained from twenty-seven cadavers at autopsy and the plasma potassium content determined as in previous experiments to get a baseline for future studies on stored cadaver blood.

The results are tabulated in table 26.

Table 26

Serum Potassium of Cadaver Cardiac Blood Obtained at Autopsy

Number	Initials, Hospital Number	Autopsy Findings	Hours Postmortem: Blood Obtained	Hours Postmortem: Serum Separated	Serum Potassium: Mg. per Cent	Serum Potassium: M.eq./L
1	J.M. 534998	Rheumatic endocarditis	13	46	54.7	13.1
2	T.G. 531679	Carcinoma of stomach	5 1/2	12	59.2	15.4
3	J.G. 37890	Tuberculous leptomeningitis	5	24	60.9	15.5
4	C.V. 37862	Glioblastome of brain	12	--	64.5	16.5
5	M.H. 547187	Hyperthyroidism	3	--	70.0	17.9
6	M.L. 538189	Congenital heart disease	9	--	70.4	18.0
7	H.LaC. 11647	Fracture of skull	8	15	72.0	18.4
8	C.O'K. 37736	Cerebral abscesses	6	8	73.5	18.7
9	A.R. 487166	Uremia	8	14	76.6	19.6

Table 26

10	L.H. 522385	Chronic myeloid leucemia	7	34	77.4	19.8
11	M.F. 289643	Carcinoma of breast	2 1/2	45	77.7	19.8
12	I.B. 548449	Placenta praevia	5	11	80.8	20.6
13	G.H. 545832	Carcinoma of stomach	--	--	87.2	22.3
14	R.R. 433652	Uremia	7	--	97.0	24.7
15	B.T. 552683	Gangrenous appendicitis	48	49	98.0	25.0
16	W.R. 367436	Carcinoma of sigmoid	11 1/4	12	98.3	25.1
17	H.R. 451845	Lymphosarcoma	6 1/2	56	98.9	25.3
18	S.F. 250743	Uremia	7 1/2	--	110.0	28.1
19	W.H. 284144	Rheumatic endocarditis	11 1/2	19	118.0	30.1
20	M.S. 37623	Meningioma	12	38	118.0	30.1

Table 26

21	G.M. 520112	Subacute bacterial endocarditis	14 1/2	34	118.6	30.3
22	J.S. 543474	Carcinoma of colon	7 1/2	--	128.0	32.7
23	V.E. 552258	Carcinoma of stomach	13 1/2	22	141.0	36.6
24	R.R. 294654	Rheumatic endocarditis	6	--	160.0	40.9
25	K.S. 549302	Cerebral thrombosis	8 1/2	155	166.0	42.4
26	K.B. 546147	Venous angioma of brain	18	67	175.0	44.7
27	R.M. 545415	Carcinoma of kidney	22	--	180.0	46.0
		Average	11	36 3/4	101.0	35.8

Comment

When the results are compared with the normal values and those observed in cardiac blood at death (table 27) the average is seen to be about six times greater than the former and 3.5 times greater than the concentration found in the latter.

A glance at Figure 2 for comparison willshow that the rate of diffusion is much more rapid in cadaver blood.

Several causes for such increased rate of change suggest themselves:

1. The temperature of the body at death and the failure to immediately refrigerate which means that the blood for a period is stored at room temperature or above, not at 2 - 4°C, the temperature at which blood is usually reduced as soon as it is withdrawn for storage. Shamov (1929) in his earliest work pointed out the marked effect the temperature has on the blood and other tissues.

2. The natural autolysis that takes place in dead tissue.

3. The formation of ammonia as the result of breakdown of body proteins.

Table 26A

Plasma Potassium of Normal Venous Blood*

Sixty Donors

Average (mean)	17.2 mg. per cent
Median	17.2 mg. per cent
Range	13.5-21.5 mg. per cent
Standard deviation	0.33 mg. per cent
Coefficient of variation	1.9 per cent

*This group comprised 50 males and 10 females. Each value represents the mean of two aliquots of the original sample, 0.5 ml.of plasma being used.

Table 27

Plasma Potassium of Human Blood*

Number of samples analyzed	Source of sample	Plasma potassium expressed as Milligrams per cent		Plasma potassium expressed as Milliequivalents per liter	
		Average	Range	Average	Range
73	Venous blood from normal young adults	17.2	13.5-21.5	4.4	3.5-5.5
13	Cardiac blood removed at death	29.7	24.0-38.0	7.6	6.1-9.7
27	Cardiac blood removed at autopsy**	101.0	54.7-180.0	25.8	14.0-46.0

*Centrifuged at 2,000 r.p.m. for 1 hour after which the plasma or serum was immediately separated from the cells.

**The average time of separating the serum from the cells after death was 31.5 hours.

The figure for each determination represents the mean of 2 aliquot samples.

Conclusions

1. The rate of cell disintegration of blood in a cadaver as measured by the rate of potassium diffusion is much greater than that of fresh blood stored at once at $4^{o}C$.
2. Blood from cadavers may present changes at the time of withdrawal comparable to those found in fresh stored blood 3-5 days old.

CHAPTER IV

Part X

Ammonia Changes in Preserved Blood

The high potassium values found in the observations on cadaver blood raised two questions. The first: Are these values true? The second: If true, why so high?

It has been known for a long time that in the cobaltinitrite method of potassium determination any ammonia present would be quantitatively precipitated as ammonium calbaltinitrite along with the potassium. In a deed body, where autolysis goes on at a rapid pace even at low temperatures, the possibility of breakdown of protein substances with release of ammonia is not only plausible but very probable.

To rule out this probability as a factor several cadaver bloods were examined for their ammonia content. The method was imperfectly worked out at the time but with crude methods the highest ammonia value found was 1.3 mg. per 100 cc.. This small quantity even if it were falsely read with the potassium precipitate, could not, it was concluded and this conclusion was concurred in by Doctor Jacques, account directly for the marked increase in cadaver plasma potassium values over the levels found just at death.

Ammonia, even in minute quantities might, however, have the same effect on the potassium diffusion of a red blood cell in plasma as it does on the Valonia in sea water. Jacques (190) has shown that if the concentration of ammonia in sea water is raised by 0.0001 molar there occurs a rapid exit of potassium from the Valonia as though the cell were injured. There has been a very strenous effort on the part of nature it seems to prevent a concentration even this great in human plasma for Conway (1935) has shown that there is less than one part of ammonia in 30,000,000 parts of blood.

To determine just what part ammonia does play in the changes observed in stored blood a series of experiments were planned.

Experiment (1)

Method

To ascertain the effect of ammonia on potassium diffusion in vitro a relatively stable ammonium compound was chosen to begin with, NH_4CL, and the bloods were kept at about body temperature to simulate a little closer the actual state of a patient at death.

To one of two similar portions of citrated blood 0.133 grams of ammonium chloride was added to make a concentration

of approximately 0.01 molar solution. These flasks were suspended in a water bath kept at 38°C throughout the experiment and were undisturbed except for the taking of the samples. The results are tabulated in table 28.

Interpretation

One obvious fact is that the control blood cells gave up potassium at a markedly increased rate when compared to bloods stored at 4°(Part I). On the other hand, these figures offer some difficulty when an interpretation is attempted in regard to the ammonia-potassium relationship. If the ammonium chloride diffused equally throughout the entire fluid mass approximately 10 to 12 milligrams of ammonia nitrogen were added to each 100 cc. of plasma. If this quantity was precipitated as potassium then the difference recorded between the control and ammoniated blood is due to precipitation of the ammonia as cobaltinitrite, and not due to an increased rate of loss of potassium from the cells. If this be so, the difference due to ammonia nitrogen actually present would remain relatively constant, therefore any increase in the difference between the control plasma potassium values and that in the ammoniated blood, which is greater than that of the first determinations would seem to be due to a true increase in diffusion.

Table 28
Effect of Ammonia on the Rate of Changes in Preserved Blood

Temperature 38°C.

Date	Time in hours from phlebotomy	Potassium as milligrams per cent in plasma of citrated blood		
		Control	Ammoniated blood	Difference between specimens
3/18/39	0	20.9	--	--
	2	19.6	30.6	11.0
	4	20.0	29.0	9.0
	6	20.3	31.7	11.4
3/19/39	8	21.9	33.2	11.3
	15	23.0	40.0	17.0
	18	27.2	43.9	16.7
	23	30.9	52.8	21.9
3/20/39	31	40.4	55.7	15.3
	39	79.9	98.1	18.2
3/21/39	71	121.0	120.0	1.0

The next question remains: Is there a gradually increasing amount of ammonia in stored blood; and if so, what levels does it reach on successive days?

To answer this question, Dr. Margaret E. Smith in this laboratory spent several months working out an accurate technique for determining the minute amounts of ammonia-nitrogen in human blood, based on the method of Conway (1935). The latter was able to show that the normal content of free NH_3 in venous blood was either zero or below the analytical level, but when shed into an open vessel a rapid rise occurred in the first five minutes. In a later publication (1939), he divided this free NH_3 into three categories, namely: (1) the "alpha" NH_3 which originates immediately after shedding and apparently arises from adenosine; (2) the "beta" NH_3 which was shown to be derived after a series of stages from adenyl-pyrophosphoric acid; and (3) the "gamma" NH_3 which appears to come either directly or indirectly from adenyldeoxyribonucleotide, or vegetable adenylic acid.

He concluded that from available evidence it appears that most, if not all, of the NH_3 forming in sterile blood collected by open s edding is derived finally from adenosine.

Experiment (2)

Method

Blood was collected in air at room temperature with no

special precautions from ten healthy donors and its ammonia-nitrogen content determined as quickly as possible after withdrawal. Analysis was carried out by the "method of suspended absorption, with the special micro-diffusion unit" (83). The results are tabulated in table 29.

This range is comparable to that reported by Conway and Cooke (84) for this age of plasma after shedding and will be used as baseline values for the following experiments:

Experiment (3)

To determine the rate of increase in the ammonia of blood stored for actual transfusions, samples were taken from twenty flasks at the time the blood was given the patients and analyzed.

Results

The bloods varied in age from 15 to 209 hours (1 to 9 days) and in milligrams of ammonia nitrogen per 100 cc. of plasma from 0.31 to 0.82. There was a sharp rise for the first ninety-three hours (four days), then an almost constant level for the next five days. These results are graphically presented in figure 16.

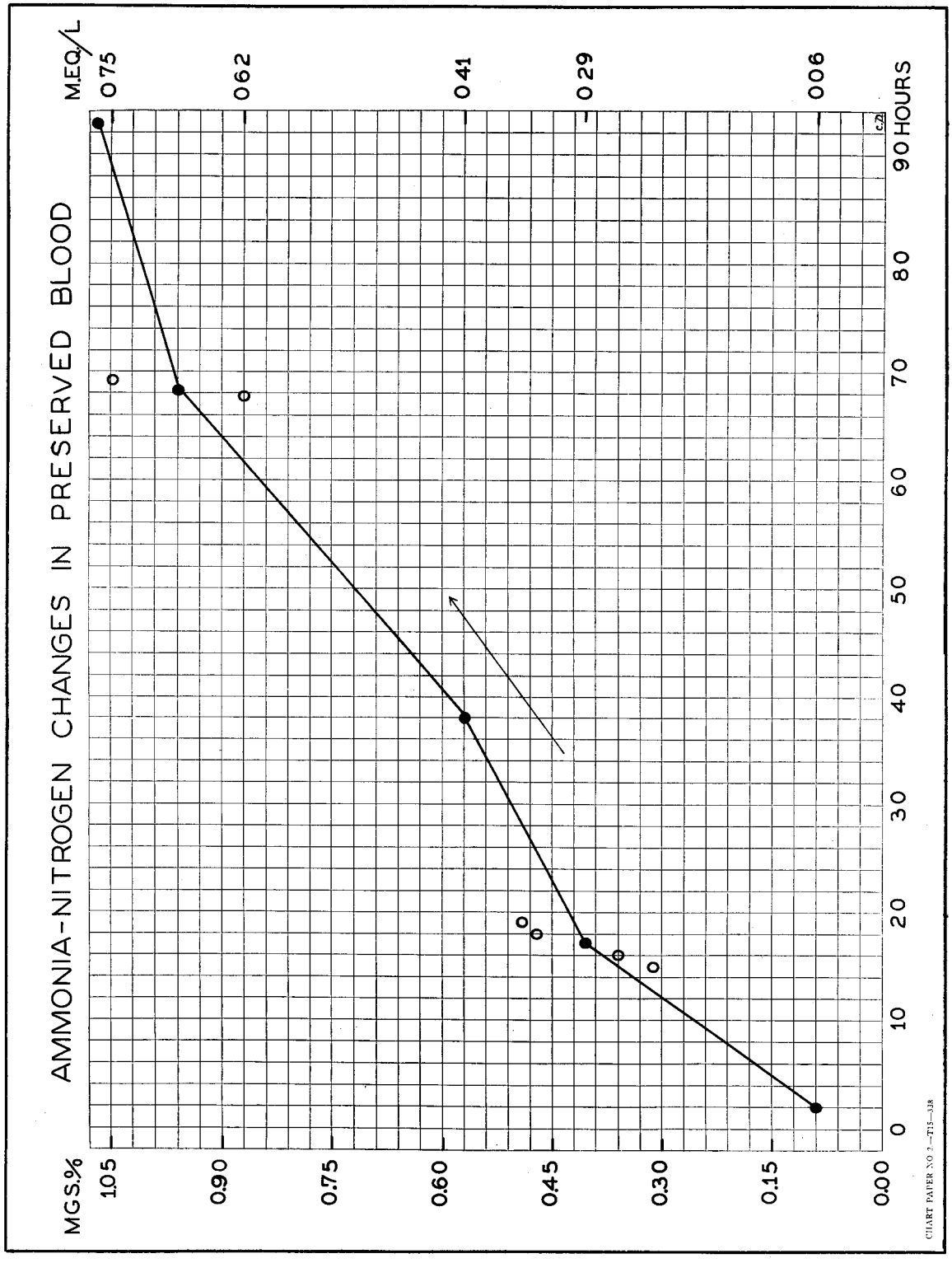
Discussion

In this series of experiments no attempt was made to

Table 29
Plasma Ammonia Content of Recently Shed Venous Blood

Number	Age in Hours	Milligrems Per Cent	Milliequivalents Per Liter
504	1	0.081	0.058
502	2	0.131	0.094
541	2	0.109	0.078
533	2	0.074	0.053
532	2	0.085	0.061
557	2	0.022	0.016
542	2	0.131	0.094
509	3	0.059	0.042
534	3	0.076	0.054
507	3	0.042	0.030
Average	2.2	0.081	0.066

FIGURE 16



Data from table 32

Human blood in vivo contains less than one part of ammonia in 32,000,000. When shed in air there is a rapid increase in the first few minutes, then a slower rise.

follow the precaution suggested by Conway to the effect that all bloods for ammonia determinations should be drawn under carbon dioxide to prevent the rapid loss of this gas when blood is drawn into an open flask. The purpose was rather to establish the values under actual operating conditions in a hospital transfusion service and check, if possible, the effect of the ammonia of blood so drawn on the rate of cell disintegration as measured by the rate of potassium diffusion. The results from the data presented are rather inconclusive. All that can be said is that there was a gradual rise in the ammonia-nitrogen content of the plasma of citrated blood as the blood became older.

Conclusions

1. Citrated bloods kept at 38°C show changes in its plasma in three days comparable to those observed in blood stored at 4°C on the fifteenth day.
2. There is a constant rise in the plasma ammonia of preserved blood, marked in the first few minutes, continuing at a rapid rate the first four days to reach a level of approximately one milligram per cent at which level it remains until the tenth day.
3. Blood, kept at body heat, to which ammonia chloride

has been added shows a greater rate of potassium diffusion for the first three days of storage. In the presence of ammonia, the possibility of false high potassium values as a result of precipitation of ammonia cobaltinitrite along with the potassium cobaltinitrite is pointed out.

CHAPTER IV

Part XI

Sodium Changes in Preserved Blood

In the intact human organism, when some disturbance causes a rise in potassium, there is often seen a concomitant lowering of sodium values. Such a condition has its best illustration perhaps in severe Addison's disease (124).

In preserved blood it has been demonstrated that there is a rapid diffusion of potassium from the cells into the plasma. If this is interpreted to mean that the permeability of the cell membrane has changed sufficiently to allow one cation to pass out, it seems reasonable to expect another to pass in.

To test this concept, a series of analyses was run to determine the changes in the sodium content of preserved blood.

Preliminary Analyses

Approximately similar quantities of fresh venous blood were collected from the same donor in three separate hematocrit tubes. In the first tube there was no anticoagulant; the blood promptly clotted. In the second tube there was placed exactly one milligram of sodium heparin. In the third tube, 0.5 cc. of

3.5 per cent sodium citrate was mixed with exactly 4.5 cc. of blood.

Sodium determinations were then done on the serum from the first tube and the plasma of the second and third tubes after centrifugation at 2,500 r. p. m. for one hour.

Method

Sodium was precipitated as uranyl zinc sodium acetate according to the method of Butler and Tuthill (69) with certain refinements added by Miss Tuthill, now a member of this laboratory staff.

Results of Preliminary Analyses

(1) Serum Na	321.7 mg. %	139.8 M.eq./L.
(2) Heparinized plasma Na	324.5 " "	141.0 " "
(3) Citrated plasma Na	398.4 " "	169.0 " "

To correct for the sodium added in the form of sodium heparin it was necessary to run analyses on the heparin and actually determine the amount of sodium in one milligram of the substance since the formula is not known accurately enough to calculate its content. Checks were excellent and the averaged results showed 0.172 milligrams of sodium in each milligram of sodium heparin. The hematocrit showed a cell volume of 47.4 per cent, plasma volume of 52.6 per cent and total volume of 4.81 cc.. Plasma volume, therefore, equals

0.526 x 4.81 equals 2.53 cc.. The sodium added to the plasma equals $\frac{0.172 \times 100}{2.53}$ equals 6.8 milligrams per cent.

To correct for the sodium added in the form of sodium citrate, the following steps were necessary. $Na_3C_6H_5O_7$-5.5H_2O has a molecular weight of 357 of which sodium forms $\frac{69}{357}$ or 19.3 per cent.

Of the total 5.0 cc. in the tube, 4.5 cc. was blood, 2.25 cc. plasma and 0.5 cc. of 3.5 per cent sodium citrate of which 17.5 x .193 or 3.38 milligram were sodium. This quantity in 2.75 cc. of plasma citrate mixture equals $\frac{3.38}{2.75}$ x 100 equals 122 mg. per cent sodium added to the sodium already present. This amount subtracted from the observed reading and corrected for dilution is as follows: 389 - 122 x $\frac{275}{225}$ equals 328.9 milligrams per cent or 142.8 milliequivalents.

Corrected values, therefore,on the same blood were as follows:

(1) Serum sodium	321.7 mg. %	139.8 M.Eq./L.
(2) Heparinized plasma Na	317.9 " "	138.1 " "
(3) Citrated plasma	328.9 " "	142.8 " "

These observations showed that fairly accurate results could be obtained using plasma instead of serum. An attempt was made to establish a series of normal values, using each of the anticoagulants.

Experiment (1)

Blood samples were collected from each of eight normal individuals in hematocrit tubes which contained exactly one milligram of the sodium salt of heparin. Plasma sodium was determined as in the preliminary analysis. The results are recorded in table 30.

This range of values compares closely with those obtained in the Medical Laboratories in a long series of serum sodium determinations.

Experiment (2)

In a manner similar to experiment (1) the range of normal values was checked on citrated blood plasma. The results are listed in table 31.

Experiment (3)

A sample of blood was obtained from each of nineteen transfusion flasks at the time of the transfusion. An attempt was made in collecting the blood to obtain accurately one part of 3.5 per cent sodium citrate to nine parts of blood. At times this was not quite possible. The plasma was analyzed from each flask for ammonia, potassium, and sodium content. The corrected results are tabulated in table 32. Figure 17

Table 30
Plasma Sodium in Normal
Heparinized Bloods

Number	Milligrams Per Cent		Milliequivalents Per Liter
	Observed	Corrected	
1	327.0	320.1	139.1
2	326.5	319.9	139.0
3	329.9	323.4	140.5
4	322.9	316.0	137.5
5	323.3	316.7	137.8
6	328.9	322.0	140.2
7	324.5	317.9	138.2
8	324.5	317.9	138.2

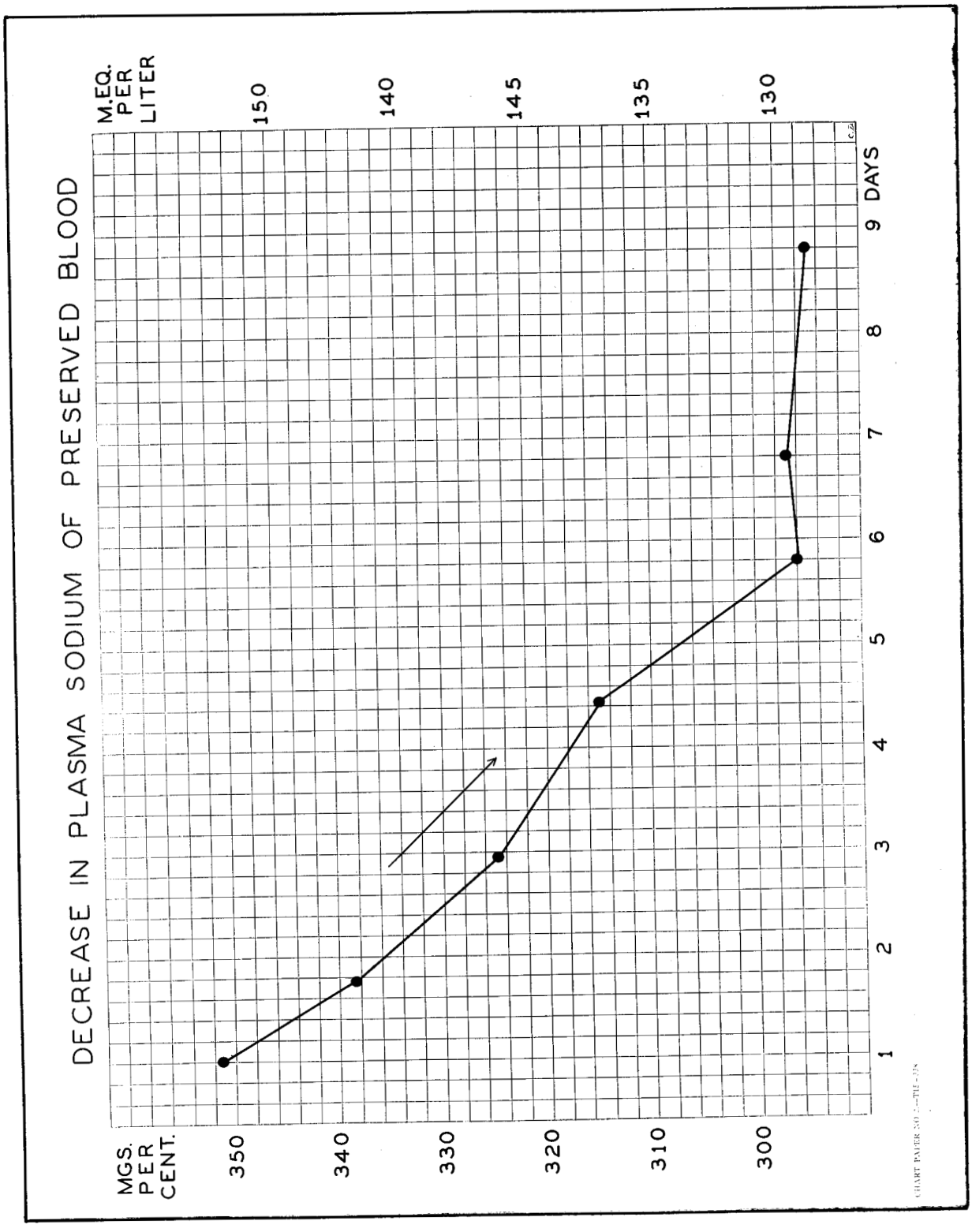
Table 31
Plasma Sodium in Normal
Citrated Bloods

Number	Milligrams Per Cent		Milliequivalents Per Liter
	Observed	Corrected	
1	380.2	317.3	137.8
2	380.5	318.0	138.0
3	396.6	337.8	146.6
4	379.6	316.8	137.6
5	378.5	315.5	137.0
6	373.9	309.8	134.5

Table 32
A Comparison of the Rate of Change
in Plasma of Ammonia, Sodium, and Potassium

Serial Number	Initial Donor	Age of Blood in Hours	Ammonia-Nitrogen		Sodium		Potassium	
			Mg. Per Cent	M.eq./L	Mg. Per Cent	M.eq/L	Mg. Per Cent	M.eq./L
504	A	15	0.34	0.24	299.5	129.7	31.2	8.0
584	J	15	0.28	0.20	313.9	136.3	37.5	9.6
555	P	16	0.41	0.29	317.2	137.7	30.9	7.9
502	A	16	0.32	0.23	317.7	137.9	21.5	5.5
586	Y	18	0.49	0.35	314.8	136.7	34.0	8.7
532	B	18	0.46	0.33	305.2	132.5	32.6	8.3
507	B	19	0.49	0.35	320.7	139.2	32.6	8.3
509	N	20	0.30	0.22	319.2	138.6	27.4	7.0
533	J	38	0.57	0.41	297.8	129.3	34.0	8.7
542	C	68	0.87	0.62	283.5	123.1	55.5	14.2
484	P	69	1.05	0.75	--	--	83.7	21.4
592	W	93	1.08	0.77	261.0	113.3	133.0	34.1
541	L	116	0.99	0.71	285.6	124.0	132.0	33.8
557	K	140	0.92	0.66	251.9	109.4	95.8	24.5
483	H	140	1.06	0.76	--	--	123.0	31.5
534	Y	163	1.15	0.82	256.7	111.4	126.0	32.1
611	L	163	0.81	0.58	250.1	108.6	136.0	34.7
549	W	184	1.09	0.78	260.4	113.0	142.0	36.4
593	P	209	1.00	0.72	251.2	109.0	135.0	34.4

FIGURE 17



Data from table 32. This curve was constructed from figures which were uncorrected for the sodium in the preservative. The true range of values was from 320.7 to 250.1 milligrams per cent, or 139.2 to 108.6 milli-equivalents.

was drawn from results which have been uncorrected for sodium in the citrate solution.

Discussion

The question of the permeability of the red blood cells to soluble anions and cations has long been a burning and unsettled one. Under normal conditions it is practically impossible for ions to cross this membrane but it has been shown that an increase in CO_2 tension or change in fluid medium (273) or change in pH (290) will markedly change this state of selective permeability. Blood stored in anti-coagulants which have a certain toxic action do lose potassium from the cells at a constant rate and the above series of observations suggests that sodium enters the cells at a very rapid rate for about five days and then reaches a state of equilibrium.

The extreme values recorded show a difference of 30 milli-equivalents of sodium in one week. This, apparently, has gone into the cells. There seems no other way to account for its loss. During this same period there was a difference of 29.4 milliequivalents of potassium. The ammonia nitrogen increased approximately 30 per cent.

Sodium and potassium, although very similar from a physical and chemical viewpoint, functionally are so different in the human being that at times they seem to be actual

antagonists. What part these ionic changes play in the destruction of the vitality of the cell in vitro or in vivo, is not known. It does seem plausible, however, to think that a substance which could decrease the permeability of the cell wall without injury would greatly prolong the usefulness of preserved blood.

Conclusions

1. There is a rapid, constant decrease of sodium in the plasma of preserved blood.
2. The rate of decrease in sodium is roughly inversely proportional to the rate of increase of plasma potassium.
3. There is suggestive evidence that as the ammonia content of the blood increases, permeability of the cell membranes is changed. This allows freer passage of ions and they have a tendency to establish an equilibrium on the two sides of the membrane in place of the marked gradient which exists in perfectly normal cells.

CHAPTER IV

Part XII

The Effect of pH on the Permeation of Erythrocytes by Cations

The leakage of cation from cells suspended in salt solutions is a very stringent criterion of cell permeability, for the integrity of the cell is unlikely to be maintained for long under such conditions.

Gürber (273) originally reported the impermeability of erythrocytes to sodium and potassium, but Hamburger and Bubanovic (162) pointed out in 1910 that if the salt concentration of the serum is changed or the carbon dioxide tension is altered both cations will readily cross the cell membranes.

Mond (1928) working with the cells of oxen showed that marked changes occurred only when the pH had risen to at least 8, and considered all the conclusions drawn from previous work on permeability where accurate account of the pH changes had not been made as open to considerable doubt.

Conway (1935) showed that only by collecting venous blood under CO_2 could the true ammonia content be determined, for there is a sharp rise in the ammonia content of the plasma which terminates at the end of about 3-5 minutes, then passes

into a slow ascent for several days. This increased ammonia increases the pH of the blood.

Jacques (1938) has shown that even minute quantities of ammonia in sea water will greatly change the permeability of the cell membrane of the sea alga, Valonia machrophysia, Kutz, to sodium and potassium.

Maizels (1935) has shown that moderate shift in pH causes increased permeation, probably by physical changes, but marked shift either side of 7.0 causes changes in permeability as a result of actual cell destruction. He also pointed out that in glucose solutions, CO_2 at first diffuses out of the cell more rapidly than base bicarbonate, hence renders the external solution acid; thereafter potassium and bicarbonate diffuse out and return the fluid to a neutral or slightly alkaline condition. The addition of small quantities of sodium chloride to the solution, it is thought, delay de-adsorption or maintain the charge on the cell surface and so prevent loss of cation. The question of CO_2 transport was well reviewed by Houghton in 1935.

All of these findings are of extreme importance in the actual practice of transfusion of preserved blood for only through an understanding of such an interplay of forces can one hope to prepare eventually an anticoagulant which will maintain osmotic balance, constant pH and biological properties of such blood.

In Part I it was noticed that among all the preservatives tested only that containing glucose and salt in the proportions suggested by Rous and Turner (1918) completely prevented hemolysis. At the time it was recorded that the pH of blood kept in such a solution was lower, nearer neutral than in the other bloods, but no significanee was attached to it. When Doctor Scudder, as a result of his association with Doctors Osterhout and Jacques, became greatly interested in the effect of CO_2 on the blood, it was noticed that the pH of those bloods taken under CO_2 likewise had values at times lower than those of freshly drawn blood.

All investigators who have reported results on blood stored in glucose have shown a sharp fall in the pH of the blood (Chapter II) and each has noted the superiority of such solutions in preventing hemolysis. In this laboratory tests run on distilled water showed a pH of 5-6.5, the ten per cent glucose solutions were found to have a pH of 3.75 to 4.25; the 5 per cent glucose solutions, 4.5 - 5; and the sodium citrate, an average of 7.6. Some commercial preparations of sodium citrate have been observed to have a pH of over 8, especially in the more concentrated forms.

Expecting to find a complete parallel between the degree of hemolysis and the pH, a series of determinations was done on bloods which happened to be on hand at the time.

The results were as follow:

Blood	Hemolysis	pH
In Lithium Citrate	+	7.19
In Sodium Citrate	++	7.73
A.T.S. in citrate	+	7.51
Placental blood, E	+	7.81
" " V	++++	7.86
" " G	0	7.59
In sodium citrate	+++	7.73

It is obvious that with such uncontrolled material and with so little correlation in the results, no conclusion can be drawn. More controlled work was planned as follows:

Methods

In each of eight experiments, blood was obtained from a different individual in the usual manner; one half of the sample was drawn into an atmosphere of carbon dioxide while the control was collected in air. On the samples so taken, from four to six determinations of the ammonia, potassium and sodium were made at intervals during a two-week period.

In each experiment, ten 50 cc. centrifuge tubes with a diameter of 2.5 centimeters were used as containers. Carbon dioxide from a cylinder, filtered through sterile cotton, was introduced into the bottom of five centrifuge tubes to displace the air. To each were added 2.5 cc. of 3.5 per cent sodium citrate and 22.5 cc. of blood. For the carbon dioxide experiments, the blood was admitted directly tothe bottom of the centrifuge tubes; in the controls, the blood was allowed

to flow in at the top. Both sets were closed with sterile rubber stoppers, sealed with paraffin, and placed in a refrigerator at 4° centigrade. At intervals during a two-week period, a tube and its control were removed from the refrigerator and centrifuged. The supernatent plasma was then drawn off for analyses.

The ammonia was determined by the method of Conway (83), the potassium by a modification (389) of the argenticobaltinitrite method of Breh and Gaebler (57), and the sodium by the method of Butler and Tuthill (69). Determinations of pH were made on six of the ten samples, using the glass electrode of MacInnes (272). Measurements of ammonia, sodium, pH, and the development of color in the potassium determinations were done in a constant temperature room, 20.5° ± 1° centigrade.

Results

The results in all of the experiments were consistent. In each, the concentration of ammonia in the blood taken under carbon dioxide was lower, the rates of potassium and sodium changes slower and the pH nearer neutral at the end of the experiment. A typical set of findings is tabulated in table 33 and graphically shown in figure 18.

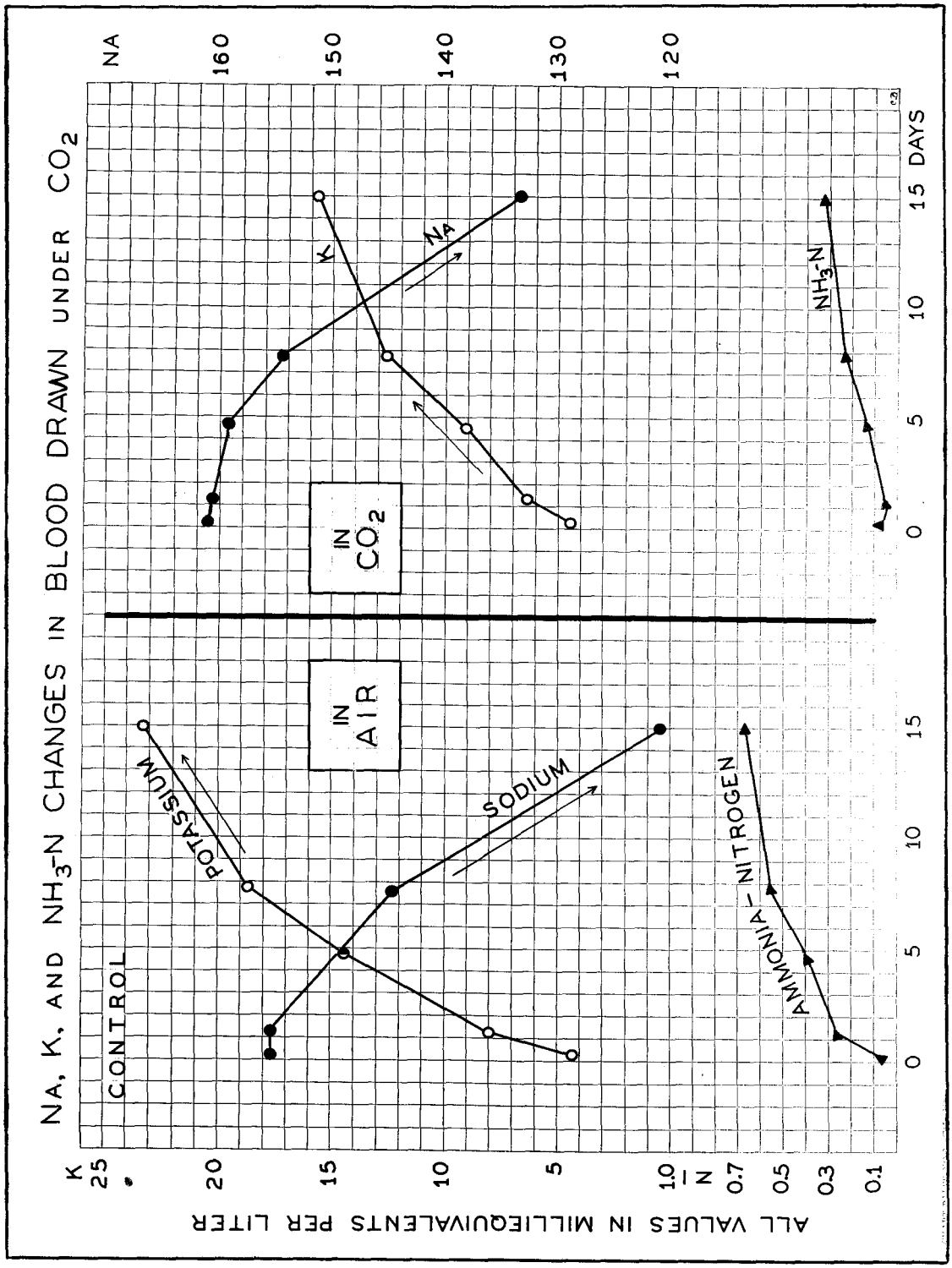
Discussion

The concentration of ammonia in the control beginning

Table 33
Effect of Carbon Dioxide on Sodium,
Potassium, Ammonia-nitrogen, and pH Changes
in Preserved Blood

Date	NH_3 - N				Na				K				pH	
	Air		CO_2		Air		CO_2		Air		CO_2		Air	CO_2
	Mg.%	M.eq./L.	Mg.%	M.eq./L.	Mg.%	M.eq./L.	Mg.%	M.eq./L.	Mg.%	M.eq./L.	Mg.%	M.eq./L.		
9/11/39	0.10	0.07	0.01	0.01	322.1	139.8	337.4	146.5	17.1	4.4	17.4	4.5	-	-
9/12/39	0.37	0.27	0.08	0.06	324.0	140.7	336.0	145.9	31.3	8.0	25.4	6.5	7.76	7.48
9/16/39	0.55	0.39	0.18	0.13	302.6	131.4	333.0	144.6	56.4	14.4	34.6	8.9	7.58	7.22
9/19/39	0.77	0.55	0.30	0.21	296.4	128.7	317.0	137.6	73.9	18.9	49.9	12.8	7.65	7.31
9/26/39	0.94	0.67	0.44	0.31	276.8	120.2	312.5	135.7	91.6	23.4	62.1	15.9	7.69	7.17

FIGURE 18



Data from table 33. The sodium values as presented are uncorrected for the sodium in the preservative. Range: Sodium in control 322.1 to 276.8 milligrams per cent; in carbon dioxide, 337.4 to 312.5 milligrams per cent. All changes are less marked in carbon dioxide than in air. pH in control 7.76 to 7.69; in carbon dioxide, 7.48 to 7.17.

at .07 milliequivalents per liter rose to 0.67, an increase of 60; in the CO_2 environment it began at 0.01 and rose to 0.31, an increase of exactly 50 per cent of that seen in the control.

Sodium values decreased in the control 19.6 milliequivalents; in CO_2, 10.8 or 55.1 per cent as much.

Potassium values increased in the control 19.0 milliequivalents; in CO_2, 11.4 or 58.4 per cent as much. The pH values in CO_2 approached a normal value of seven more closely than they did when taken in air.

No measurements of the amounts of hemoglobin lost in the plasma were done in these experiments but hemolysis in the samples taken in CO_2 was less than in those taken in air.

Conclusions

Blood drawn under carbon dioxide maintains a pH value nearer neutral than blood drawn in air and is effective in retarding changes in the concentrations of ammonia, sodium, and potassium.

CHAPTER IV

Part XIII

Calcium and Phosphorous Changes in Preserved Blood

Of the six metals commonly present in living matter, calcium is the one with the greatest tendency to form insoluble salts (282). In man, for the most part, it is found to be present in the form of the phosphate or carbonate and may be divided into two forms (1) diffusible and (2) indiffusible. The diffusible portion alone is capable of existing in the ionized state and only a small portion is ever actually dissociated from its very stable salts (408).

In the blood it is found in the form of tricalcium phosphate, a relatively insoluble compound, but under the influence of carbonic acid of the plasma, it is partly converted to the more soluble calcium bicarbonate and calcium hydrogen phosphate, which may ionize to some degree in the following manner:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftharpoons 2CaHPO_4 + Ca(HCO_3)_2 \rightleftharpoons$$

$$2Ca^{++} + 2HPO_4^{-} + Ca^{++} + 2HCO_3^{-}$$

When carbonic acid tension is reduced, reprecipitation

of the ionized active forms will take place. Howland (408) has expressed this interdependence in the following manner:

$$\frac{(Ca^{++}) \times (HCO_3^-) \times (HPO_4)}{H^+} = K$$

It can be seen that the concentration of any one ion will at once produce changes in the concentration of the other ions. A reduction of CO_2 tension in the blood will lower the H ion concentration, therefore, an equivalent diminution in the calcium, bicarbonate and phosphate ions must follow. Clinically this condition is seen in alkalemia.

In preserved blood it has been observed in the preceding experiment that there is a tendency for CO_2 to escape, thereby changing the H ion concentration of the blood and causing a change in the partition coefficients for sodium, potassium and ammonia.

Ideally the bicarbonate value on the same blood should be done at the same time the calcium and phosphorous content is done. In this particular experiment mechanical and technical difficulties made this impossible, but the changes in the latter two are of interest.

Methods

Calcium was determined by the method of Clarke and Collip (78), the final titration being done against a standard sodium oxalate solution.

Phosphorous, determined as HPO_4, was done by an adaptation of the methods of Collip and Clarke (78) and Fish and Subbarow (129). Following Gamble's (134) suggestion the valence of the HPO_4 radicle is considered 1.8.

Procedure

From a voluntary donor 450 cc. of blood was drawn in air into a wide-mouthed straight bottle containing 50 cc. of 3.5 per cent sodium citrate, and divided into twelve equal portions.

On the plasma of one sample, calcium and phosphorous determinations were done on the day of collection and on the following day. Then on every second day, two tubes were removed from the refrigerator and the plasma removed from one to be analyzed. The second tube was inverted five times to thoroughly mix the blood and centrifuged for thirty minutes before the plasma was removed.

Results

The results are presented in table 34 and show the changes which took place during nine days. Each of the values is an average of at least two separate determinations by two individuals.

Table 34
Calcium and Phosphorous Changes
in Preserved Blood

Age in Days	Calcium				Phosphorous			
	Before Shaking		After Shaking		Before Shaking		After Shaking	
	Mg.%	M.eq./L.	Mg.%	M.eq./L.	Mg.%	M.eq./L.	Mg.%	M.eq./L.
0	9.2	4.6	--	--	3.6	2.1	--	--
1	8.8	4.4	8.7	4.4	3.5	2.0	3.4	1.97
3	8.9	4.5	8.7	4.4	3.4	1.97	3.4	1.97
5	8.6	4.3	9.2	4.6	3.5	2.0	3.6	2.1
7	8.7	4.4	8.7	4.4	3.8	2.2	3.6	2.1
9	9.1	4.6	9.1	4.6	3.7	2.1	4.0	2.3

Summary

1. The calcium content of the plasma of preserved blood remained constant for a period of nine days and was not influenced by moderate trauma in the form of shaking.
2. There was a slow but definite increase in the plasma phosphorous content and this increase was accentuated by trauma, especially in the oldest blood.

CHAPTER IV

Part XIV

Magnesium Changes in Preserved Blood

Magnesium is found in cells in a greater quantity than any cation except potassium. Joseph and Meltzer (214) reported in 1910 that the toxicity of the chlorides of magnesium, calcium, potassium and sodium varied in almost inverse proportion to the quantities found normally in the blood, particularly in the plasma. Since potassium constitutes approximately 0.02% and magnesium 0.01% of the blood, significant increases in plasma magnesium, according to this theory, should prove twice as toxic as potassium. To discover whether or not magnesium leaves the cells of stored blood as freely as potassium, determinations were done on a series of bloods of different ages.

Procedure

Twelve equal portions of blood preserved in a 1:9 mixture of 3.5% sodium citrate and blood were collected and for a period of nine days, plasma magnesium determinations were done before and after shaking, as in Part XIII. The method used was an adaptation of several methods (58, 78, 129). The results are presented in table 35.

Table 35

Magnesium Changes in Preserved Blood

Age in Days	Magnesium in Milliequivalents per Liter Before Shaking	After Shaking
0	2.3	—
1	2.3	2.35
3	2.5	2.4
5	2.6	2.4
7	2.5	2.5
9	2.4	2.4

Conclusion

1. Magnesium diffuses out of red blood cells into the plasma of stored blood at a very slow rate.
2. Moderate trauma does not increase appreciably this loss.
3. The actual increase in magnesium at the end of nine days' storage appears to be too small to play any part in toxic manifestations following transfusions with preserved blood.

CHAPTER IV

Part XV

Changes in the Total Electrolyte Structure of the Plasma of Preserved Blood

Having observed the changes in potassium, sodium, ammonia, calcium, phosphorous, magnesium and pH of stored blood, the question of the maintenance of total ionic balance in bloods gradually growing older loomed as an unknown well worth investigation.

To this end freshly drawn blood from the blood bank was set aside at approximately monthly intervals for four months. At the end of this time determinations of the plasma sodium, potassium, calcium, magnesium, bicarbonate (CO_2 combining power), chloride, phosphate and pH were made on the several samples.

Complete studies on the sulphates, organic acids and proteins could not be carried out at this time so that the final answer to the complete acid-base balance has not yet been attained, but the cation picture is complete and the anion picture far enough along to clearly point out trends.

There is not complete unanimity of opinion concerning the equivalent values to be assigned to the sulphate organic acid and protein components on the acid side of the equation.

In this Institution we have followed the values established in the Department of Medicine as a result of many studies by Atchley, Loeb and Gutman. A comparison of the figures with those of Gamble of Harvard is present in table 36.

Methods

Potassium, sodium, calcium, phosphorous and magnesium determinations were done as in preceding experiments.

The chlorides were determined by the method of Van Slyke (394), the carbon dioxide and oxygen by the method of Van Slyke and Neil (395), and pH determinations were done on the MacInnis and Longsworth glass electrode potentiometer (272).

The bloods which were examined on the fifth, sixty-eighth and one hundred and seventeenth days of preservation were stored in narrow-waisted dumbell shaped flasks which contained 50 cc. of 3.5 per cent sodium citrate in distilled water to 450 cc. of blood. All but one of these flasks were inverted to thoroughly mix the cells and plasma, before the sample was removed for centrifugation and removal of the plasma for analysis. The values in thelast column were obtained from the plasma of a blood one hundred and seventeen days old, which had not been disturbed during this entire period.

Table 36
Acid-base Composition
of Fresh Blood Plasma

(Expressed in Milliequivalents per Liter)

Base			Acid		
Ion	Gamble	Gutman	Ion	Gamble	Gutman
Na^{+}	142	142	HCO_3^{-}	27	28
K^{+}	5	5	Cl^{-}	103	104
Ca^{++}	5	5	HPO_4^{--}	2	2
Mg^{++}	3	2	SO_4^{--}	1	1
			Org.Ac.	6	1
			Protein	16	18
Total	155	154		155	154

The twenty-one day old blood was kept in a wide-mouthed flask while the ninety-three day old blood, column six from the left, was collected in a Baxter Transfuso-vac bottle which contained 70 cc. of 2.5 per cent sodium citrate in physiologic saline.

All values have been corrected for dilution and added sodium or chloride. No attempt has been made to establish the equivalent value of the citrate added. This, it is hoped, can be done when the attempt is made to complete the acid side of the picture.

Results

The results are tabulated in table 37.

Discussion

When it is considered that the bloods used in this series of experiments were obtained from six different individuals, were not accurately measured as to content of sodium citrate and blood and, at the time of analysis, had stood in the refrigerator from one week to four months, the balance in the cations is striking.

The greatest changes, as expected, are seen in the very soluble and diffusible sodium and potassium ions. In the jar which contained the added sodium chloride (ninety-three days old) the final sodium value was the lowest, the

Table 37

Changes in Total Cation Structure
in the Plasma of Preserved Blood

(Expressed in Milliequivalents per Liter)

Cations	Normal Gutman	Age in Days 5	21***	68	93*	117	117**
Na^{+}	142	128.4	106.3	119.0	89.7	105.2	130.7
K^{+}	5	28.2	35.0	29.2	54.2	40.6	28.8
Ca^{++}	5	5.3	5.5	5.7	5.7	5.3	5.4
Mg^{++}	2	1.9	2.5	2.2	2.4	2.7	2.1
Total	154	157.8	149.3	149.0	152.0	153.8	167.0

Changes in Anion Composition
in the Plasma of Preserved Blood

Anions							
HCO_3^{-}	27	16.6	11.9	15.4	12.4	10.7	16.5
Cl^{-}	103	99.3	99.7	100.5	89.0	99.7	107.0
HPO_4^{--}	2	1.9	6.0	5.2	8.2	7.5	3.4
pH	7.3	7.31	7.34	7.28	7.10	7.25	7.24

* Baxter Transfuso-vac. ** Undisturbed plasma. *** Wide mouthed flask.

potassium value the highest, as though the added sodium had increased the concentration gradient and forced more of the cations into the cells when the permeability had been changed sufficiently to allow free passage.

Calcium in these bloods stored for longer periods acted similarly to that in bloods stored for shorter periods and showed relatively little change, nor did thoroughly mixing a one hundred and seventeen day old blood increase the plasma calcium content.

Magnesium, as the second largest constituent of the cells, might have been expected to show a greater outward diffusion. Apparently, it forms compounds within the cell which are more stable, hence less readily ionized and slower to diffuse out.

The average number of milliequivalents of the totals in the six bloods studied amounts to 153.8 compared to the control normal value of 154.0. Very true indeed is Claude Bernard's conception of the constancy of the milieu interieur (29).

The alkalie reserve of the plasma as measured by the CO_2 combining power of the blood decreases with age. No exact relation can be established from these data to conclude that it varies directly with the pH.

The chloride content diminishes but not to the extent the sodium does. Two things are striking in the table; the

the low value at the end of ninety-three days in the flask which contained 70 cc. of normal saline at the time the blood was taken, and the high value of the plasma chloride at the end of four months in the flask which was left undisturbed.

The phosphate gradually increased in the plasma with the increasing age of the blood even in the undisturbed state, more so when agitated but never so great as the rate at which potassium is lost from the cells.

The ph of stored blood, though quite variable in the early days of its storage as shown by previous experiments, has a tendency to equalize conflicting forces and approaches its normal level after several months.

The total quantity of negative ions in these six bloods ranged from one hundred to one hundred twenty-six milliequivalents per liter, an average of approximately one hundred and sixteen.

Having shown that the total positive ionic content remains constant, that the ph varied very little, that the protein changes very little (356), the quantity of negative ions necessary to complete the acid-base balance must be supplied by increases in the organic acid ion content. There is evidence (Chapter III) that a part of this may come from the increasing quantities of lactic acid formed by the process of glycolysis. This is a factor yet to be integrated with

the above facts to complete the picture of the complete acid-base composition of the plasma of stored blood.

Summary

In the plasma of bloods stored from five days to four months, the following changes were observed:

1. Potassium increases.
2. Sodium decreases.
3. Calcium remains practically constant.
4. Magnesium increases slowly.
5. Bicarbonate decreases.
6. Phosphate increases, particularly following trauma.
7. Chlorides decrease in plasma intimately mixed with the cells, remain constant or slightly increase when left undisturbed.
8. The pH decreases at first then gradually approaches the level of normal blood.
9. The total positive ionic content remains constant, in spite of great variations in the plasma content of the various cations.
10. The observed loss of total negative ions suggests that balance is maintained by a gradual increase in the organic acid ion component. This factor is to be determined.

CHAPTER IV

Part XVI

General Summary and Interpretation of Experimental Studies

1. Plasma Potassium Increase

There is a daily increase in the amount of potassium present in the plasma of preserved blood, beginning at the time the blood is drawn, mounting to twenty-five per cent of the total potassium content of the red blood cells at the end of ten days, and reaching fifty per cent at the end of thirty days, at which time the concentration begins to become equalized inside and outside of the cells.

This suggests that bloods stored over long periods of time should be withheld or carefully given in clinical conditions associated with hyperpotassemia such as is found in cholera (351), intestinal obstruction (358), severe burns (357), renal (50) and hepatic (41) insufficiency, typhoid fever, influenza, pneumonia (232), hypoparathyroid tetany (153), the collapse state of Addison's Disease (3, 124, 275, 388) and in other diseases of the endocrine system associated with disturbances in salt metabolism. (21, 163, 166, 266, 275, 276). There is evidence to suggest that its inconsiderate use may be dangerous in hemorrhage and shock (356), the field of its greatest usefulness.

2. Hemolysis as a Criterion of Toxicity

The degree of hemolysis cannot be used as an index of potassium loss or toxicity of blood. Hemolysis may be completely prevented by the use of a glucose-saline-citrate preservative but no preservative yet found will prevent loss of intracellular electrolytes. These changes are the most sensitive indicators of the changes in permeability of the cell membrane and of the vitality of the cell. No good practical index of toxicity is known.

3. Toxicity of Potassium

The potassium ion has an almost specific action on the heart and peripheral vascular tree, producing on injection disturbances varying from diminished cardiac out-put to immediate arrest, depending on the rate of injection. Reasoning by analogy from data obtained from animal experimentation, it would require between 3,000 and 5,000 cc. of thirty-day old blood, given at a fairly rapid rate of injection, to kill a healthy man of average size.

This suggests that preserved blood is safe if ordinary clinical judgement is exercised.

4. Red Blood Cells and Hemoglobin

Erythrocytes remain almost constant in number for fifteen days in any of the usual preservatives, diminish

about twenty per cent by the end of the first month in citrate solution, and remain intact for periods exceeding a month in citrate-saline-glucose mixtures with maintenance of full oxygen carrying ability. They become gradually more fragile. First signs of hemolysis under good storage conditions appear between the twelfth and twentieth day in citrate, only after thirty days in glucose citrate. Hemoglobin content remains constant in the total specimen, being found in measurable quantities in the plasma about the twentieth day and amounting to fifteen to twenty per cent of the cell content at the end of thirty days, a decrease which, in part, is reflected in the twenty per cent loss in the mean cell diameter of the red cells.

Banked blood, therefore, theoretically may be used from the point of view of erythrocyte and hemoglobin content for fifteen days with prospect of excellent results, for thirty days if stored in glucose with fair results. Because of the increasing fragility of the cells and the possibility of jaundice, it is advisable to use only bloods of less than ten days' storage.

5. White Blood Cells

Leucocytes as a whole disintegrate rapidly, the polymorphoneuclear neutrophiles, in particular, so that at the end of two or three days it is doubtful whether any are capable

of function. Because of the close association of the white cells with the phagocytic, bactericidal and immune properties of the blood, it is suggested that where transfusions are indicated because of their antibody content fresh blood be used or at least blood stored for not over three days.

6. Prothrombin

The prothrombin content is not markedly reduced for at least ten days and may remain at levels higher than those associated with bleeding tendencies for four months. Blood stored for many days, therefore, may be used in cases of prothrombin deficiency with the expectation of good results. Earlier reports from this clinic showing rapid loss of prothrombin concentration were due to the use of old brain extract in carrying out the Quick test. When the work was repeated with freshly extracted rabbit brain as the source of thromboplastin substances, the fall in prothrombin levels was very gradual.

7. Platelets

The thrombocytes fall rapidly to a level between 10,000 and 80,000 within the first three days of storage and then remain fairly constant depending on the anticoagulant used.

In cases of essential thrombocytic purpura, it would

seem advisable to use fresh blood or blood which had been stored for less than three days to obtain maximal therapeutic results.

8. Effect of Trauma

The effects of trauma on blood cells are magnified by increasing age. Blood which is to be exposed to the trauma of transportation should be sent to its eventual destination as soon after withdrawal as possible, in order to insure the least possible damage to the increasingly fragile cells.

9. Ideal Container for Blood

The rate at which diffusion of intracellular electrolytes takes place into the plasma varies approximately as the diameter of the container at the interface of the container. Blood, therefore, should be stored in a flask with a narrow waist to diminish the rate of diffusion, a narrow neck to prevent changes in the plasma over a widely exposed area, and the air space at the top should be at a minimum to prevent splashing and damage to the cells on movement. The material should be heat resistant, alkalie free and should not be covered by such foreign substances as paraffin for prolonged storage.

Such a container has been designed and tried out in this laboratory with gratifying results.

10. Placental Blood

The cell volume of placental bloods is approximately fifty per cent higher than that of the mothers at term and twenty-five per cent higher than that of the average healthy adult. Its protein content is lower. The cells disintegrate at a rate similar to that of adult bloods. The danger of contamination is greater and the amounts obtained from each placenta seem to vary with the technique of the operator, but once obtained, upon experimental grounds, it is equal to or better than adult blood when compared volume for volume. It must be grouped and cross-matched with the same care as blood obtained from any other source.

11. Cardiac Blood at Death

The concentration of potassium in the hearts' blood of animals obtained at death following the intravenous injection of lethal doses of potassium chloride, or following induced shock, was uniformly higher than that found in human beings at death.

This suggests that there is danger in translating the data from animal experimentation in terms of human resistance to a toxic substance. Man may be more sensitive to the toxic action of a poison like potassium than dogs or cats. In using bloods stored for long periods with marked deteriorative changes, a large margin of safety should be allowed.

12. Cadaver Blood

Blood from cadavers may present changes at the time of collection comparable to those found in donor blood stored for three to five days. The rate of cell disintegration as measured by the rate of potassium diffusion is greatly in excess of fresh donor blood stored under similar conditions. There is an element of safety in the use of cadaver blood stored without anticoagulant. Those bloods collected from persons dying of severe infections or wasting diseases will clot, while only bloods from relatively healthy individuals who have died acutely will undergo fibrinolysis and be suitable for use.

There are certain psychic inhibitions to the use of such blood but the matter is worthy of real consideration.

13. Effect of Heat

Citrated blood kept at 38°C. shows changes in three days, comparable to those observed in blood stored at 4°C. after fifteen days. The danger of infection is greatly increased.

All bloods should be reduced to a temperature of three to five degrees C., as soon as drawn and stored in a refrigerator which is free from mechanical shaking or movement, used for storage of blood alone.

14. Ammonia and Cell Permeability

Freshly drawn blood rapidly loses carbon dioxide and allows a sharp rise in the ammonia content of the plasma. This, in turn, increases the permeability of the cell membrane and allows more rapid leakage of cellular electrolytes, a condition detrimental to the vital properties of the blood.

Blood drawn under carbon dioxide does not show these rapid changes, therefore, is kept in a better state of preservation for longer periods of time. When bloods are to be used soon after drawing the added precaution of collecting it under increased carbon dioxide tension does not seem worthwhile; but if bloods are to be stored for longer periods, especially if they are to be transported, the extra precaution seems well worthwhile.

15. Plasma Sodium Decrease

There is a constant decrease in the sodium content of the plasma of preserved blood. The rate at which this takes place is roughly inversely proportional to the rate at which the plasma potassium increases. Both are evidence of a break-down in the selective permeability of the cell membranes.

There is no way of completely preventing this shift of electrolytes in preserved blood at the present time.

16. Shifts in pH Concentration

The permeability of cell membranes is markedly affected by wide shifts of pH from that of the blood in the body. Some citrate solutions are too alkaline and cause rapid change in pH with consequent increased rate of electrolyte displacement.

When carbon dioxide tension is maintained, the pH changes are less. When glucose is added to the preservative, there is at first a tendency for the fluid to become more acid due to glycolytic processes, when this process slows down there is a shift back towards the alkaline side and a restoration of the status quo ante.

Blood drawn under carbon dioxide and preserved in glucose maintains a pH value close to neutral and prevents the changes which follow marked shifts in hydrogen ion concentration.

17. Calcium and Phosphorous

The calcium content of preserved blood remains at practically a constant level for periods as long as four months.

Phosphorous shows a very slow but steady increase in the plasma and this is accentuated by trauma.

The changes in neither appear to be of great practical significance.

18. Total Cation Structure

Potassium goes steadily and rather rapidly up in the plasma of preserved blood and magnesium increases much more slowly, while the phosphorous content increases so slowly that it may be considered just a tendency. Sodium values steadily fall and calcium remains constant. The sum of all of the positive ions when expressed as equivalents of hydrogen remain constant, always equalling the total for fresh plasma in spite of the very marked difference in distribution between the cells and the plasma.

19. Anion Changes

The alkalie reserve as measured by the carbon dioxide combining power decreases with the increasing age of the blood, while the phosphate which to a large degree is an intracellular constituent, gradually increases as the increased permeability of the cell membrane allows easier passage of both cations and anions. The chlorides diminish but not to the extent the sodium does.

20. General Conclusion

Blood preserved for periods not exceeding ten days, insofar as could be determined in these investigations, is safe and for most purposes should give results when used for transfusions comparable to those following the use of fresh blood. Its rapid loss of white cells precludes its use in

cases where bactericidal properties or antibodies usually associated with white cells are desired. It would seem less effective in cases of thrombocytemenic purpura and bleeding due to prothrombin deficiency. Its use in large quantities would seem contraindicated in patients suffering with diseases associated with hyperpotassemia but even in such cases its moderate and intelligent use should lead to no untoward effects.

CHAPTER V

PRESBYTERIAN HOSPITAL BLOOD BANK

Part I

The Establishment of the Bank

Following preliminary discussions between Mr. John Bush, Superintendent of the Hospital, and Doctor Fordyce B. St. John, Doctor David C. Bull, and Doctor John Scudder, on November 28, 1938, the Chairman of the Medical Board, Doctor J. Bentley Squier, at the request of Mr. Busch, asked the Board to endorse the appointment of a committee to investigate the advisability of the use of a blood bank; the committe to be composed of:

Doctor Fordyce B. St. John, Chairman
Doctor A. R. Dochez
Doctor David C. Bull

At a meeting on January 30, 1939, the committee reported their findings and recommendations to the Medical Board. A brief resumé of that report is as follows:

The committee appointed to study the problem presented by the use of stored blood for transfusions has approached its review by:

1. A study of the practice of this method.
2. Observations on the results of the experimental work, no in progress and sponsored by the Blood Transfusion Betterment Association of this city.

A review of the literature reveals the paucity of material of value to date.

The use of placental blood is not included in this study.

Finally, your committee has not taken up the economic phase of the problem except to include the observations of this factor by those practicing the method.

Practice

A questionnaire was sent to a selected group of clinics using the stored blood method of transfusion. Six clinics were chosen because of their experience, and your committee's knowledge of the men working in them.

The following questions were asked, after an explanatory note:

1. How long have you used the blood bank system? Is this the only system in use? If not, in what percentage of cases?
2. What is the source of supply of the blood bank - patients, friends, family or professional donors, and about the percentage of each?
3. What percentage of reactions have you had since instituting this system, and how does this compare with reactions resulting from the use of fresh blood, direct or indirect?
4. What are the criteria used in classification of reactions?
5. Have there been deaths, thought to have been due to transfusions?
6. In brief, how does the blood bank system function?
7. Is it possible to give an approximate idea of the saving from an economic standpoint?
8. Has there been any objection, serious or otherwise, on the part of donors?
9. Any medico-legal trouble?

This communication was sent to:

1. The First Surgical Division of Bellevue Hospital Attention of the Director
2. The Graduate Hospital of the University of Pennsylvania Attention of Dr. Walter E. Lee
3. The University of Minnesota - Attention of Dr. Wagenstein
4. The Cook County Hospital in Chicago - Attention of Dr. Kellogg Speed
5. The Mayo Clinic, Rochester - Attention of Dr. Balfour
6. The Mt. Sinai Hospital of New York - Attention of Dr. Neuhof

The responses from four of these clinics are incorporated in this report because of their informative value.

University of Pennsylvania Graduate Hospital

The system had been in use fourteen months; the source of supply 99 per cent the family; less than 1 per cent reactions in 920 transfusions compared to 10 to 12 per cent before; no deaths; no medico-legal trouble; plasma removed after seven days; has earned a very definite place.

First Surgical Division, Bellevue Hospital, New York City

Blood bank in use five months; only system used; 1100 transfusions; 95 per cent of donors, relatives or friends; reactions 4.7 per cent; saving the city $2,500 to $4,500 per month.

Cook County Hospital, Chicago

Inaugurated March 1937; practically only method used; source, friends and relatives 95 per cent; reactions, first year 12.2 per cent, second year 5.9 per cent; deaths, three in 4000 transfusions.

Mt. Sinai Hospital, New York City

Began June 1938; 938 transfusions; 13.9 per cent reactions, exactly the same as in 1000 transfusions of fresh blood; stored blood changes very little.

Based on a study of the foregoing observations, and others on the use of stored blood, your committee has reached the following conclusions:

1. Time element. The method has been used a sufficient length of time to justify serious considerations.

2. The technique has been developed apparently to the point where no unusual and dangerous reactions are to be anticipated. The possibility of potential danger from the jaundice and potassium phenomena, however, has not been eliminated. This requires further study as do other factors.

3. The indications of the results to date warrant comparison with past methods. We further believe that this method of transfusion may well replace fresh blood transfusions in the future in a number of conditions.

RECOMMENDATION:

On the basis of the above conclusions, your committee recommends that this method be given an experimental trial in this Clinic.

Dr. Fordyce B. St. John,
Chairman

This report was accepted by the Board and it was "Resolved: That an experimental trial be made of a blood bank in this hospital; that the present committee should carry on, institute its installation and have supervision over all its details."

Doctor John Scudder was appointed to direct the laboratory and experimental aspects of the project and Doctor Charles R. Drew to manage the bank and direct clinical investigations.

CHAPTER V

Part II

Organization of the Blood Bank

1. Location and Schedule

Actual operation began on August 9, 1939. The following announcement was sent to the house staff on the following day.

PRESBYTERIAN HOSPITAL
EXPERIMENTAL BLOOD BANK
Ward L - East. Room #43

To the Hospital Resident Staff

Gentlemen:

The experimental blood bank went into effect on August 9th for a trial period of four months.

Purpose:

(1) To ascertain the safest method or combination of methods to preserve human whole blood for purposes of transfusion.

(2) To check clinically certain laboratory observations which bear directly on the indications and contraindications for the use of preserved blood.

(3) To estimate, if possible, the economic advantages of a blood bank in this hospital.

Problem:

(1) To ascertain more accurately the physical, chemical and biological changes which take place during the storage period of bloods kept in various types of

containers and with different anticoagulants.

(2) To establish, if possible, the relationship of these changes to the effectiveness of such blood in specific clinical conditions and to the unfavorable results sometimes seen in blood transfusions.

Time:

6:00 P.M. Monday, Wednesday and Friday.

Place:

Vanderbilt Clinic - C Floor, northern-most corridor (Surgical Diagnostic Clinic)

Donors:

To report to admitting desk by appointment on above stated days.

They must be fasting.

They will be typed for phlebotomy to insure bleeding only compatible donors during the trial period. Five bloods a night will be taken.

Types of Cases:

For the present the "bank" will not be a full functioning part of the hospital service. The cases must be elective so that the studies may be complete and accurate. Emergencies do not permit such studies, hence are to be kept at a minimum. The right to select cases is reserved by the "bank".

Routine Procedure:

I. To make appointments for donors, the house staff should get in touch with Miss Stoddart, Extension 7489, or Miss Sargent, Extension 7045; or a requisition should be left in L-43 with the (1) name, (2) diagnosis, (3) blood group of the intended recipient, (4) ward, and (5) possible number of donors.

If the appointments are not filled for the next "bank night", the donors may then be notified by the ward staff of the time to come into the clinic.

II. To obtain blood from the "bank", the ward must have made a deposit or incur a debit which must be made up by sending in additional donors. Such blood may be obtained from Miss Stoddart or the physician in charge by presenting a requisition on Form S-40 completely made out with type and amount of blood desired, age, diagnosis, race of patient, etc.. Blood from the intended recipient should be in the bacteriology laboratory on the morning of the intended transfusion for cross matching. It would facilitate the experimental work to have all "blood bank" transfusions started at 2:00 P.M.

Reactions:

Any reactions in the form of rise in temperature, chill, nausea, vomiting, urticaria, shock or convulsion should be reported by the ward staff to the "blood bank" staff.

To run this experiment with any modicum of success we shall need and, therefore, earnestly ask for the cooperation and good will of the attending physicians, the resident staff and nurses.

Sincerely yours,

D. C. Bull
J. Scudder
C. R. Drew

2. Personnel

The Staff at the opening, including those who were to carry on laboratory procedures as well as those concerned with the actual mechanics of running the bank, was composed of the following:

Full-time nurse	Miss Helen Stoddart
Part-time nurse	Miss Margaret Barnett
Chemist	Miss Elizabeth Tuthill
Laboratory technician	Miss Eunice Thompson

Laboratory technician	Mr. Josiah Lasell
Research part-time chemist	Dr. Margaret E. Smith
Part-time secretary	Mrs. Margaret Nelson
Nurses aid	Mrs. Thelma Owens
Secretary	Miss Mary Sargent

In March, 1940, Mrs. Martha McKenna was added as a second nurses aid so that the unit could function for sixteen hours a day. Mrs. Lenore Drew filled in for Mrs. Nelson who voluntarily left because of increased home duties, and Dr. Kingsley Bishop to aid in the investigations on plasma.

3. Health Department Regulations

To operate a "blood bank" in the State of New York and in New York City, certain regulations had to be complied with in accordance with Section 106 of the Sanitary Code as amended on March 14, 1939.

The rules governing the examination of donors, the collection and testing of bloods for storage, and the keeping of records were incorporated into the routine of the Presbyterian Hospital Blood Bank.

4. The Typing of Bloods

The vast majority of all fatal accidents related to the practice of transfusions have been due to mistakes in

grouping or cross-matching (98, 359, 385, 406, 411).

To obviate the possibility of error in this regard, it was decided to group the donor by the finger tip slide method (80) before the blood was drawn from the donor. A sample of venous blood is also sent to the laboratory to have the grouping checked and at the time of transfusion the donor's cells and serum are cross-matched against the serum and cells of the recipient. This triple check is felt essential to complete safety.

When intragroup reactions are suspected, an additional check is advised in the form of the following test (405):

Two small tubes are used. Tube (1): Two drops of the patient's serum are mixed with one drop of donor's cell suspension. Tube (2): Two drops of the patient's serum are mixed with one drop of the patient's cell suspension. The tubes are put in ice water for five minutes, then centrifuged while still cold. The tubes are gently shaken and read both macroscopically and microscopically.

Reading: (1) If neither tube shows clumping, the donor is compatible. (2) If both show reaction, an auto-agglutinin is probably present, and providing the bloods are of the same group, the donor can be used without danger. (3) If tube (1) shows agglutination and tube (2) does not, the donor is incompatible.

In the presence of autoagglutination it is always wise

to inject 20 cc. of the blood, wait twenty minutes for possible reactions and then proceed slowly with the transfusion.

5. Routine and Experimental Procedures

When the donor presents himself to the secretary and host at the blood bank, his name, address, sex, color and other information of a statistical nature are recorded on a form and his serial number is assigned, then his blood group is determined and a complete physical examination performed before the phlebotomy is done with a number 13 Lewisohn needle, after novocain local as anaesthesia, and a small nick in the skin with a number 11 scalpel to prevent carrying skin into the tissues.

The experimental procedures, the clinical follow up, and the hospital release are recorded on one form. Such a form is included here.

Many types of containers have been used, rigorous care is taken in the preparation of all apparatus, each patient is followed after transfusion, the temperature being taken each hour for six hours then every four hours for twenty-four hours, all subjective as well as objective changes being recorded by some member of the blood bank staff after personal inspection of the patient. In this way many small urticarial whelps, slight rashes and mild subjective

changes have been recorded which otherwise would have been missed.

No great detail of the technique and investigations will be attempted at this time.

BABIES HOSPITAL
INSTITUTE OF OPHTHALMOLOGY
NEUROLOGICAL INSTITUTE
PRESBYTERIAN HOSPITAL
SLOANE HOSPITAL FOR WOMEN
VANDERBILT CLINIC

History and Physical Examination

UNDERLINE INSTITUTION USING FORM

ASSOCIATED WITH
NEW YORK STATE PSYCHIATRIC INSTITUTE
COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS & SURGI
COLUMBIA UNIVERSITY SCHOOL OF DENTAL & ORAL SURG

Name Date

Address Serial Number

Age Sex Color

Group Kline Last Meal

Pulse Temperature R. B.P.

History of { Syphilis, Malaria, Allergy, Polyomyelitis, Scarlet Fever, Measles, Septicaemia } Before / After

Skin
Mouth
Pharynx
Heart
Lungs
Abdomen
Liver
Spleen
Rectum
Genitalia
Lymph Nodes

Evidence of Heart Disease, Hyperthyroidism, Allergy, Tuberculosis, Venereal or other communicable diseases. Infection of teeth or gums with supperative lesions. Drug addiction.

.....M.D.

Hospital Release

I release and forever discharge.....
(Institution)

and such surgeons and physicians and their successors from all claims and demands whatsoever which I, my heirs, executors or administrators have or may have against it or its successors by reason of the giving of blood for transfusion to which I have submitted or am about to submit, and any consequences resulting directly or indirectly therefrom.

IN WITNESS WHEREOF I have hereunto set my hand and seal this.....

day of....., 19.....

.....(Seal)

In the presence of:

Donor

Serial Number
Name
Date
Group { 1 Immediate / 2 Check / 3 Crossmatch
Wassermann
R. B. C. 1.
W. B. C. 1.
Differential 1.
Hb. 1.
Hem. 1.
W. B. Sp. Gr.
Pl. Sp. Gr.
Pl. Proteins
Container
Amount of Blood Taken
Anticoagulant
Intended Recipient
Relation of Don. to Rec.
Hemolysis in Sample Given
Pl. K. 1. 2.
Pl. Na 1. 2.
Culture
Blood Used
PH of Blood 1. 2.
Reaction of Donor to Phlebotomy
Other Investigations

Recipient

Serial Number Unit
Name Age
Date
Group
Age of Blood
Diagnosis
Amount of Blood Given

R. B. C.	1.	3.
	2.	4.
W. B. C.	1.	3.
	2.	4.
Differential		
Hb.	1.	3.
	2.	4.
Platelets	1.	3.
	2.	4.
Hem.	1.	3.
	2.	4.
Pl. Sp. Gr.	1.	3.
	2.	4.
Pl. Proteins	1.	3.
	2.	4.
W. B. K.	1.	3.
	2.	4.
Pl. K.	1.	3.
	2.	4.
Cell K	1.	3.
	2.	4.

Oscillograph Tracing
Visual
Film
Course on preceding days
Febrile
Afebrile
Temperature immediately before transfusion
Reaction

Jaundice	Hematuria	Temp. alone
Rash	Hemoglobinuria	Temp. with chill.
Shock	Petechiae	Chilliness without temperature
Nausea	Vomiting	
Convulsion		Back pain

Remarks

CHAPTER V

Part III

Donor Statistics

These statistics are based on the first four hundred acceptable donors, beginning August 9, 1939, to February 23, 1940.

The vast majority were relatives or friends of the patients for whom they came in, or at least friends of a friend of the patient.

The data is presented in table 38.

Discussion

Approximately ten per cent of all donors have been rejected because of physical unfitness, past history of communicable disease, or physical findings suggesting communicable disease. The blood of approximately 1.8 per cent of the donors accepted had positive Wassermann tests and were rejected.

Table 38
Donor Statistics

Grouping		Number 400	Per Cent
Sex			
Females		25	6.24
Males		375	93.76
Ages			
Oldest	68		
Youngest	15		
Average	34.2		
Groups			
O I		195	48.7
A II		122	30.5
B III		72	18.0
AB IV		11	2.7
Color			
White		328	82.0
Colored		71	17.7
Yellow		1	.2
Relation of donor to eventual recipient			
Relative		32	8.0
Friends		11	.2
Strangers			91.8
Wassermann			1.8
Rejected			10.0

CHAPTER V

Part IV

Recipient Statistics

Statistics for the recipients are based on the first four hundred transfusions. These were given between August 10, 1939, and February 27, 1940, and were divided into groups as follows:

O I	49.9 per cent
A II	32.1 per cent
B III	15.2 per cent
AB IV	2.8 per cent

These four hundred transfusions were given to three hundred and forty-three patients. The fifty-seven patients having two or more transfusions accounted for one hundred and thirty-five of the total. The largest number to any individual was sixteen. This man had a large echinococcus cyst of the liver removed and continued to bleed. His life was saved on at least two occasions by the speed with which he was transfused during severe hemorrhage but he finally succumbed.

Indications For Transfusion

The indications for transfusion are divided into six main categories.

(1) Hemorrhage, due chiefly to ectopic pregnancies, accidents, operations or peptic ulcers.

(2) Secondary anemia. The bulk of these transfusions were for obstetrical cases, chronic noninflammatory disease on the medical wards, or preoperative patients on the surgical wards.

(3) Blood dyscrasias. Included here are the leukemias, purpuras, agranulocytic angina, aplastic anemias and erythroblastic anemias.

(4) Prophylactic or therapeutic transfusion in the operating theatre. The vast majority of the cases were transfused during the course of the operation or given blood because of an incipient fall in the blood pressure. A few were for shock or hemorrhage.

(5) Infection with secondary anemia. This group contains the septicaemias, chronic abscesses, cases of peritonitis and empyema.

(6) Anemia and hyproteinemia. Here transfusions were used as a source of nutrition in treating the cachexia associated with carcinomatosis, chronic nephritis or chronic gastro-intestinal lesions.

Table 39

The distribution of the types was as follows:

Indications	Number	Per Cent
Hemorrhage	24	6.0
Secondary Anemia	57	14.2
Blood Dyscrasias	25	6.2
Operations	81	20.2
Infection and Anemia	142	35.5
Anemia in Hypoprotenemia	71	17.7
Total	400	99.8

Transfusion Sets

The bloods on which studies were made were given in sets prepared by the blood bank staff, in the presence of or given by some member of the unit and followed up by the same individual.

Most of the transfusions given in the operating room were given, after preliminary straining, with the operating room sets. When the bank became more active and all bloods could not be given by the bank staff, the bloods were signed for by the interne and given with ward sets.

The distribution of sets used was as follows:

Sets Used	Number	Percentage
Blood Bank	219	54.7
Ward	111	27.7
Operating Room	30	7.5
Baxter	40	10.0
Total	400	99.9

Summary

Statistics concerning the first four hundred recipients of banked blood are reviewed.

CHAPTER V

Part V

Reactions

There were sixty reactions in the first four hundred transfusions, fifteen per cent.

There were no fatal accidents, no hemolytic crises, no prolonged rises in temperature and only six cases which did not show clinical improvement in the blood picture following transfusion.

The highest rise in temperature was 4° F, the average rise in those cases which had fever 1.5°F.

There was one accident, a coronary thrombosis in a man of 63 with advanced heart disease, carcinoma of the bladder and a purulent draining fistula from the bladder through the abdominal wall. Twenty minutes after a transfusion of 400 cc. of blood he felt suddenly faint, with a feeling of great weight on his chest; very little pain. His pulse dropped to forty per minute, and an electrocardogram taken five minutes after the onset of difficulty showed a complete heart block. He recovered.

Table 40

Reactions According to Type

	Number	Per cent
1. Rise in temperature alone	25	6.2
2. Chilliness alone	8	2.0
3. Chilliness with rise in temperature	7	1.7
4. Chill with rise in temperature	5	1.3
5. Urticaria	10	2.5
6. Jaundice	4	1.0
7. Coronary	1	.15
Total	60	14.85%

The patients who showed a rise in temperature alone without other subjective or objective signs may be further divided as follows:

Temperature Rise	Per cent
1 - 2	4.20
2 - 3	1.23
3_{+}°	0.77

Table 41

Reactions According to Blood Groups

Group	No.	Per cent
O	I 26	43.3
A	II 16	26.7
	III 13	21.7
AB	IV 5	8.3
	60	100.0

Urticaria

In six of the ten patients who had urticarial spots or wheals, there was a definite history of previous allergic manifestations, due chiefly to one or several articles of food. None was severe, most would have been missed entirely if they had not been looked for. In only one case was there troublesome itching.

Jaundice

The cases which had jaundice were given bloods nine, twelve, fourteen and eighteen days old. In three, there was no rise in temperature. In one a rise of 0.8° one hour after the transfusion; the jaundice developed the next day. In each case it quickly disappeared. The severest case had a serum bilirubin level of 3.7 mg. per cent.

Relation of Reactions to Febrile Course

The most striking observation in this study is the very close association of reactions in the form of chillness, chills and rise in temperature to a pre-existant febrile state. In 73.3 per cent of the sixty patients who had reactions of this type following transfusion, the temperature had not been constantly normal for the three preceding days. If the cases which showed urticaria and jaundice, the former due, it is felt, to food hypersensitiveness, the latter to destruction of old cells, are deleted over ninety per cent of the patients

showing febrile reactions were running febrile courses before the transfusion.

A volatile toxic substance in the blood of donors who faint?

The two worst reactions seen during this period of study followed the transfusion of fresh citrated blood taken from donors who fainted during the phlebotomy. The transfusion was stopped in one patient and after an hour was started again. The temperature had shot quickly to 106° following a severe chill after the introduction of about 100 cc. of blood. When the same blood was re-injected from the same flask, there was no reaction. This suggests that there is a volatile toxic substance thrown into the circulation of a person who faints which is capable of causing a reaction in a recipient if the transfusion is carried out at once, but which disappears if the blood is allowed to stand for a while.

Plasma Transfusions

The experience of this clinic is limited at the present time in the use of plasma transfusions. The few tried have worked well and there have been no reactions. All plasma is being removed from banked blood on the seventh day with the purpose of investigating its properties further.

Summary

In four hundred transfusions there were sixty reactions, all but forty relatively mild, for a reaction rate of fifteen per cent. The striking relation between the post-transfusion febrile reaction and the pre-transfusion febrile course is the most dominant finding. Persons hypersensitive to individual articles of diet are likely to develop urticaria if the blood contains the substance to which the patient is allergic.

Jaundice is likely to supervene when blood older than nine days old is used.

The reactions by groups parallel fairly closely the distribution of the bloods used; in this series there was some preponderance of reactions in group AB IV when considered in terms of the number used.

CHAPTER V

Part VI

Cellular and Protein Changes in Recipients Following Transfusion

(1) Normal Values

At the onset of investigations hematocrits, plasma specific gravities and plasma proteins were done on forty-eight male donors and ten females. Plasma, whole blood and calculated cell potassium contents were determined on fifty-one. Sodium values were established on ten and ammonia values on ten. The results were as follows:

Normal Values

Cell Volume by Hematocrit

Male	47.1	per cent
Female	43.2	per cent
Plasma Specific Gravity	1.0275	
Plasma Protein	7.02	Gm. per cent
Plasma Potassium	17.2	mg. per cent
Whole Blood Potassium	196.0	mg. per cent
Calculated cell potassium	401.5	mg. per cent
Plasma Sodium	322.8	mg. per cent
Plasma Ammonia		
in air	0.08	mg. per cent
in carbon dioxide	0.01	mg. per cent

(2) Effect of Transfusion of Preserved Blood on the Red Blood Cells Counts and Hemoglobin Content of the Recipients.

There were one hundred and eight complete studies including counts before transfusion, in twenty-five cases immediately after transfusion, in eighty-three cases one hour after transfusion and in all one hundred and eight after twenty-four hours. In addition forty-one follow-ups included checks at the end of one week but so many factors played a part in the changed cell counts after so long a period that the results were not amenable to interpretation, therefore are not included.

The results are as follows:

Table 42

Increase in Hemoglobin after 500 cc. Transfusion

	Number	After	24 Hours after
	25*	10.0%	11.0%
	83**	7.5%	6.0%
Total	108	8.1%	7.2%

Table 43

Increase in Red Cell Count after 500 cc. Transfusion

	25*	6.3%	8.5%
	83**	7.7%	6.2%
Total	108	7.4%	6.6%

In only six cases was there a failure to show an increased count, five of these were in cases where the count was approximately 5,000,000 before transfusion.

* Taken immediately after transfusion.

** Taken one hour after transfusion.

Table 44

Relation of Rise in Red Cell Count to the Degree of Anemia After 500 cc. Transfusion

Range (in millions)	1 hour	24 hours
1.4 - 2.0	32.0 %	27.0 %
2.1 - 3.0	12.5 %	12.2 %
3.1 - 4.0	7.6 %	7.9 %
4.1 - 5.0	-1.0 %	-1.4 %

Table 45

Relation of Age of Blood to Therapeutic Efficacy*

Age of Blood in Days	Percentage Rise in R. B. C. Counts 2.1 - 3.0		Counts 3.1 - 4.0	
	Stat	24 hours	Stat	24 hours
1	12.7	12.7	10.0	8.0
2	15.0	19.0	10.0	10.0
3	13.0	13.0	10.0	10.0
4	20.0	20.0	11.0	11.0
5 and 6	6.0	13.0	6.0	6.0
7 and 8	12.0	7.0	12.0	15.0
12	13.0	6.6	5.0	5.0

* There were too few transfusions given to patients with red cell counts less than 2,000,000, and over 4,000,000 to make the results of comparisons in this manner significant. Only the groups between 2,000,000 and 4,000,000, which formed the bulk of the transfusions are tabulated.

(3) The White Blood Cells showed an average of 5.9 per cent increase immediately after transfusion, a loss of 8.8 per cent in twenty-four hours. The results were unpredictable.

Table 46

Effect of Transfusion of 500 cc. of Blood on Cell Volume as Measured by the Hematocrit, and Plasma Proteins as Calculated from the Plasma Specific Gravity.

	Number	Before	After Stat	After 24 hours	After 1 week	
Cell Volume	116	30.8	33.2	33.2	34.4*	per cent
Protein	116	6.22	6.33	6.30	6.47	gm. per cent

This represents a 7.8 per cent increase in cell volume when the determination was done with in an hour of the end of the transfusion and the increase was sustained at the end of twenty-four hours. The cells in the blood of fifty-seven* of these patients at the end of a week showed an 11.7 per cent increase over the initial volume. The figures compare very closely with the observed 7.4 per cent increase in red blood cell count immediately after transfusion.

Discussion

There is an average increase in the red cell count, hemoglobin concentration and cell volume immediately after transfusion with preserved blood which approaches theoretical expectancy and at the end of twenty-four hours the increase is maintained at a level approximately 1.5 per cent higher than the initial values for each 100 cc. of blood transfused. When the blood is over seven or eight days old, there is a slight tendency for the values to drop on the second day which suggests that such blood is destroyed at a rate greater than that of bloods up to one week old. This fact gives a real criterion for establishing the age at which the red cells begin to give less than their optimum therapeutic result. This, it seems, therefore, would be the ideal time to remove the plasma. The percentage rise varies tremendously with the degree of anemia, being greatest in the severest forms.

The white cell response is so variable that no conclusions can be reached. In approximately one-half of the cases the counts went up, in the other half, down; the resultant, however, showing a positive balance immediately after transfusion. In twenty-four hours the white cell count is definitely lower when the whole series is considered. This fact must be interpreted in the light of the fact that 70 per cent of the patients were febrile at the time of transfusion.

The increment for proteins was very small. With the values established here as a basis of therapy, it requires at least 2,000 cc. of blood to raise the plasma protein one gram.

Summary

There is a definite rise in red cell count, hemoglobin content and cell volume equivalent to better than one per cent for each 100 cc. of blood used over the whole range of anemias. The increase may reach seven per cent per 100 cc. in persons with severe anemia and may show no rise in persons whose blood counts are approximately normal.

Changes in white cell count are indeterminate.

To raise plasma proteins one gram approximately 2,000 cc. of blood are required.

CHAPTER V

Part VII

Potassium and Sodium Changes at the Time of Transfusion in Bloods Stored in Various Types of Containers

Preservation of Blood

To test the validity of the hypothesis presented at the conclusion of the series of the experiments in Chapter IV, Part VI, bottles were blown embodying the principle of a narrow waist at the level interface between the cells and plasma, and used for the storage of blood. At the same time, bottles of other types (figures 19 and 20) were used and the changes in the rates of sodium and potassium recorded for each type for different periods of storage.

This work is still incomplete but certain results have been noted up to this time.

Method

Five types of containers were used to collect blood from voluntary donors in the Blood Bank (see figure 20).

(1) The Baxter 500 cc. Transfuso-vac with rubber stopper. This bottle has a diameter of 11 cm. outside measurement and contains 70 cc. of 2.5 per cent sodium citrate under 26 mm. of water vacuum and 500 cc. of blood.

(2) The Baxter 1000 cc. Transfuso-vac with rubber stopper. Similar to (1) except that it contains 105 cc. of 2.5 per cent sodium citrate, 600 cc. of blood.

(3) Narrow-waisted "dumbell" bottles, with ground glass stopper, devised to diminish interface area. Diameter of narrow portion 3.55 cm.. Each bottle holds 450 cc. of blood and 50 cc. of 3.5 per cent sodium citrate ($Na_3C_6H_5O_7$ 5 1/2 H_2O).

(4) Wide mouthed 1000 cc. jar with Bakelite cap. Diameter 9 cm.. Contains 50 cc. of 3.5 sodium citrate and 550 cc. of blood.

(5) Wide mouthed 500 cc. jar with Bakelite cap.. Diameter 7.7 cm; filled to the top, it contains 50 cc. of 3.5 per cent sodium citrate and 450 cc. of blood.

The number of Baxter flasks studied is too small ti include in the table, twenty-five in all. The results in these flasks were much less uniform and gave a range of values as follows:

In the 1000 cc. Baxter Transfuso-vac, the results expressed as milligrams per cent of plasma potassium ranged from 55.4 on the second day to 110 on the twelfth, but there was a rise to 182 on the fifth day and 250 on the seventh. The results were very variable.

In the 500 cc. Baxter Transfuso-vacs, the range was from 25.6 on the first day to 236 on the eleventh day with a fairly constant curve of progression.

For simplicity only the numbers for the "dumbell" flasks and the wide mouthed jars are used in the table.

All of these bloods were collected by breaking the stream at the adaptor to the needle as it lay in the vein. Each had been thoroughly mixed before starting thetrans-fusions. It is impossible, therefore, to be sure that each blood received exactly the same amount of shaking or was handled in exactly the same manner after it had been removed from the refrigerator. Every attempt was made to keep the technique uniform throughout. This is certain: the figures reported here represent the quantities of potassium and sodium that actually went into the patient with the trans-fusion. A total of one hundred and thirty-one studies were made. Each study included potassium and sodium determinations. Since only twelve were done on bloods older than ten days, in the table the extreme value found between the tenth and twenty-first day is recorded. Each figure represents the average of at least two bloods of the same age, most values are the mean of three or more determinations.

The results are tabulated in table 47.

Discussion

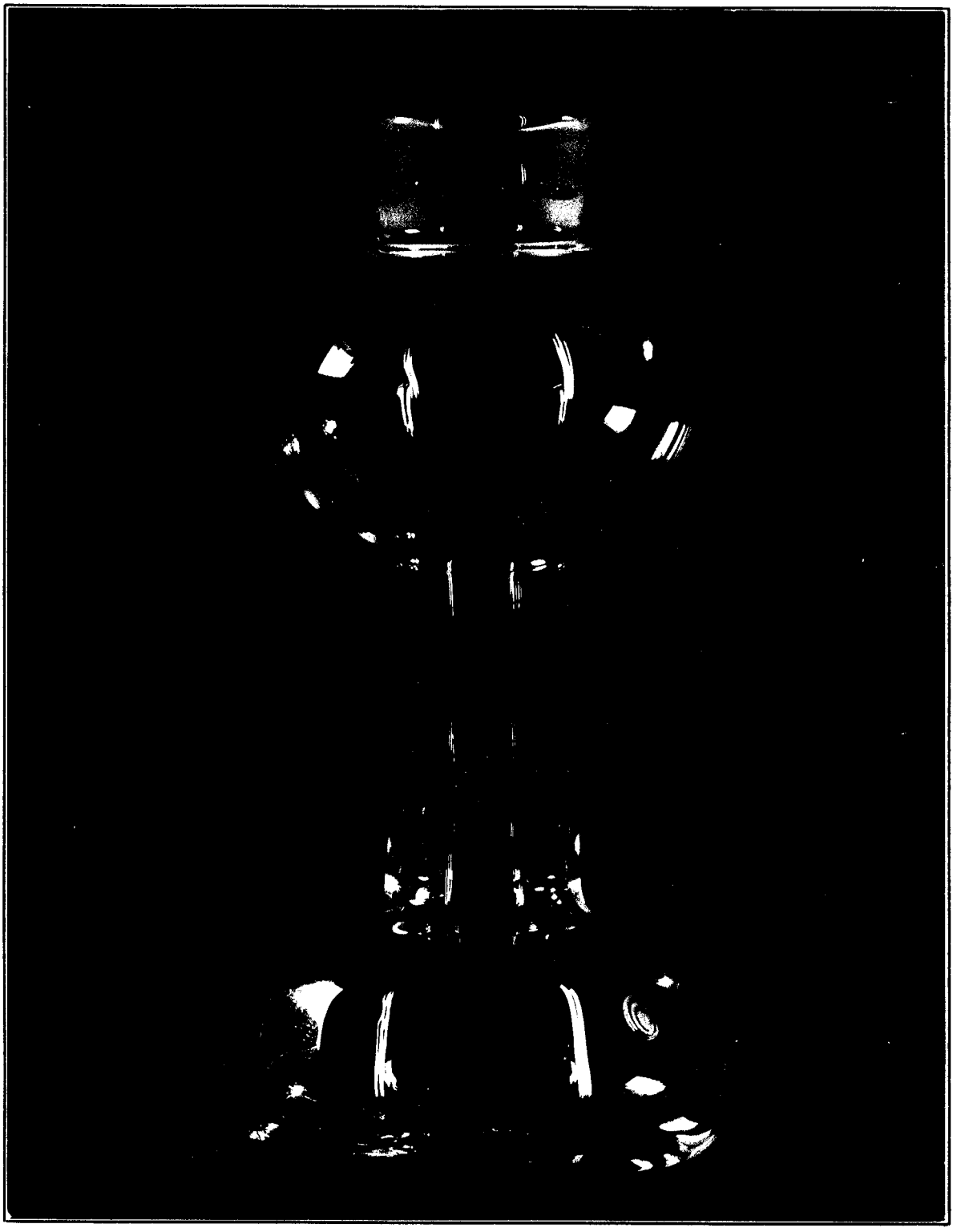
The largest amount of potassium given any patient as plasma potassium in this series was 0.7 grams. For a 50

Table 47
Potassium and Sodium Changes in
(3) "Dumbell" Flasks, (4) 1000 Cc. Jars, and (5) 500 cc. Jars

Age in Days	Plasma Potassium in Milligrams Per Cent			Plasma Sodium in Milligrams Per Cent		
	Container Number			Container Number		
	3	4	5	3	4	5
1	26.3	28.7	27.7	379.0	369.1	378.8
2	49.4	49.0	63.7	364.0	361.3	358.0
3	71.9	67.3	69.6	353.5	342.7	353.0
4	79.1	76.4	93.2	350.7	337.4	342.0
5	87.7	85.8	96.5	345.9	337.0	338.2
6	87.6	86.6	111.0	340.0	338.9	322.8
7	108.0	102.8	110.0	342.1	336.5	336.5
8	101.0	89.9	128.0	341.0	342.8	333.7
9	109.5	103.7	117.0	354.0	335.5	327.1
10	--	105.0	139.0	--	338.1	318.6
Extreme 10 - 21	130.0	141.0	151.0	328.5	314.1	316.8

A NEW TYPE OF FLASK DESIGNED TO GIVE THE MINIMAL PRACTICAL DIAMETER OF THE INTERFACE BETWEEN THE CELLS AND PLASMA

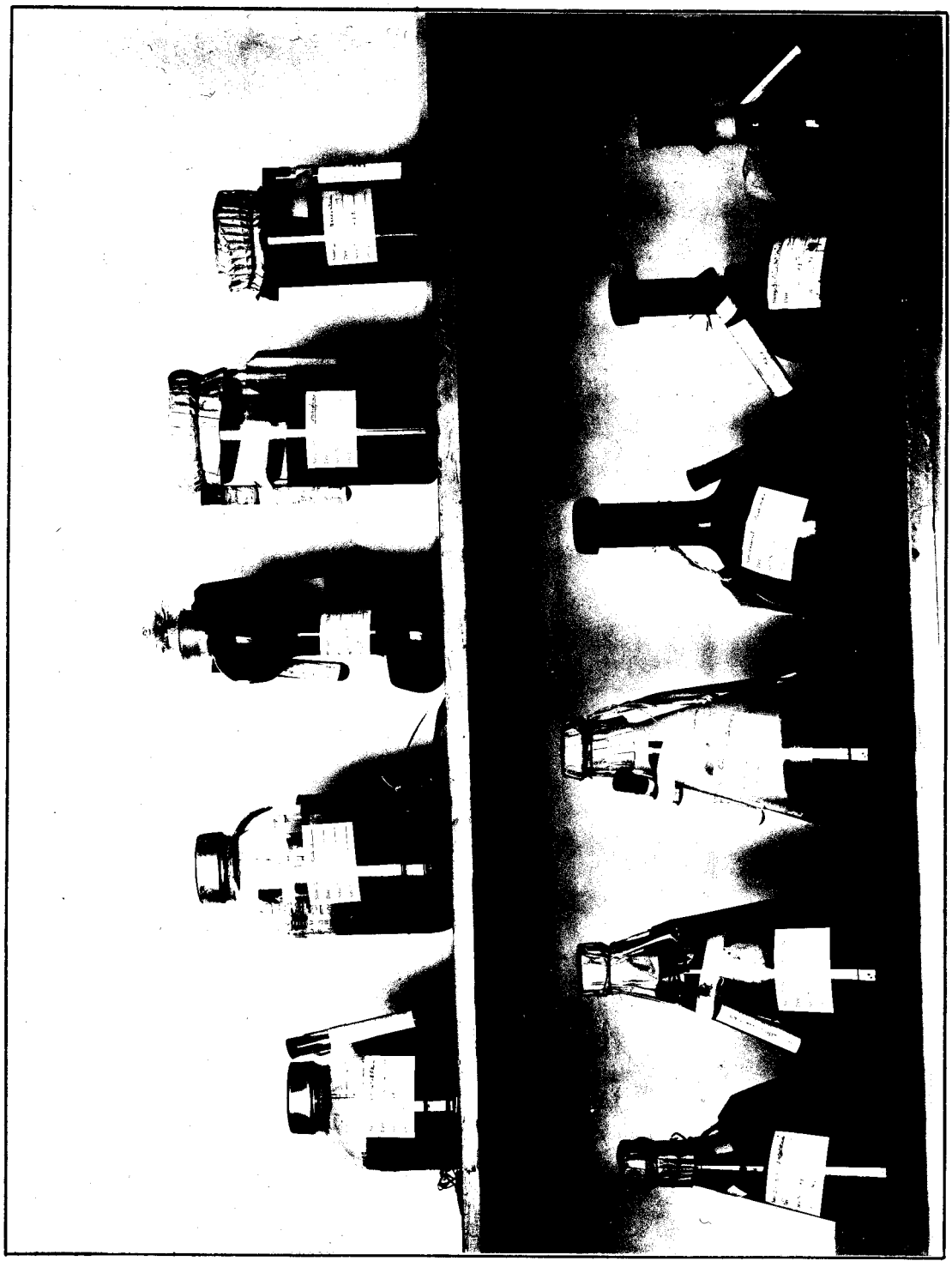
Figure 19



Approximate dimensions: Height 22 cm. Base width 12 cm. Neck and waist 3.5 cm. Width of top bulb 9 cm. Capacity: Top 230 cc. Narrow waist 70 cc. Bottom 300 cc. Total 600 cc.

CONTAINERS USED IN THE EXPERIMENTAL BLOOD BANK

FIGURE 20



Top row, left to right (1) small Baxter Transfuso-vac, (2) large Baxter Transfuso-vac, (3) "Dumbell" flask, (4) wide mouthed 1000 cc. jar, (5) wide mouthed 500 cc. Bottom row, (1) 500 cc. Erlenmeyer flask, (2) 750 cc. Erlenmeyer, (3) 1000 cc. Erlenmeyer, (4) 1000 cc. Florence flask, (5) 750 cc. Florence, (6) 500 cc. Florence.

kilogram man this represents on 0.014 grams per kilogram. In previous experiments (Chapter IV, Part III) it has been shown that it required approximately 0.140 grams per kilogram to effect a fatal issue in a dog when given at the speed of a human transfusion. If the metal has the same degree of toxicity for man and dog, there was in this case, the worst of the lot, only one-tenth of a fatal dose; i.e., a ninety per cent margin of safety.

This figure approaches the total potassium content of 500 cc. of blood. Whole blood contains on an average 200 mg. per cent of 1.0 gram for 500 cc..

Marenzi (276) has established the fact that 20 milligrams per kilogram body weight in a dog may cause death if injected within ninety seconds. If not fatal at once, the values approach normal in two minutes. The amount of potassium in 500 cc. of blood, 1.0 gram, if injected into a man of fifty kilograms at such speed would theoretically be capable of producing death.

It seems very doubtful that such a thing could happen in man, even in a hemolytic crisis, and larger transfusions by the very nature of the mechanics of infusion would require considerably longer periods of time. The unknown factor in every case, however, is the ability of the sick organism to cope with even small amounts of a toxic substance.

Contrary to all expectations the lowest plasma potassium

values on each day throughout the whole series were found in the large 1000 cc. wide mouthed open jars, almost equally as good were the values found in the dumbell shaped jars; in a poor third position the 500 cc. wide mouthed open bottle, the 1000 cc. Baxter in fourth place and the 500 cc. Baxter Transfuso-vac in last.

In previous experiments (Chapter V, Parts I and VI) it has been clearly shown that the rate of diffusion in the narrow waisted containers is markedly greater than in the large diametered containers when both are left at rest in the ice box. These results show that the changes are going on in the cells at an equal rate but the diffusion is less in the narrow tube; the undisturbed plasma remains, therefore, in much better condition, but, when the blood is thoroughly mixed, the values approach one another. It is worth noting, also, that in the case of the large jar less trauma is undergone by the cells in mixing for transfusion than in the small jar filled right to the top or in the dumbell shaped flask with the two sets of curves. Applying this hypothesis to the high values found in the Transfuso-vac containers, three sources of additional trauma present themselves. They are: (1) The force with which the blood is drawn into the bottle from the donor; (2) The glass tube which extends from the rubber stopper to the bottom of the flasks as an air intake when the bottle is inverted, which may serve in exactly the same manner as a

rod for defibrinating blood at the time the plasma and cells are mixed for use; and (3) The cells may become more fragile when stored in a vacuum. There is a clash of two concepts as the result of the above experiments. The first is: with no air space above the blood in the container, on movement there is no room for splashing, therefore, trauma is less and the cells are better preserved; the second is: if there is no air space, it requires more vigorous effort to mix the bloods at the time they are given and any advantage gained is lost at the last moment.

Since the best procedure to follow in any bank is to remove the plasma at the end of a week, the choice of method of storage and type of container used would seem to be that one which best preserved the plasma for this length of time. It was shown in Chapter IV, Part XV that plasma in the narrow waisted dumbell shaped flasks at rest at the end of four months may show potassium changes no greater than those found in the containers with wide interface areas after fifteen to twenty days.

The fall of the sodium values is in almost every case inversely proportional to the rise in potassium. Undoubtedly, as the cell membrane of the red blood corpuscle undergoes changeswhich allow cations inside to come out, it loses the ability to keep the cations outside from coming in. As the

bloods become older the membrane loses its vital, selective powers and acts more and more as a simple semi-permeable membrane.

Conclusions

1. For prolonged storage of blood, the best container is one which retains the plasma in the least changed condition. A container embodying princples which accomplish this to a degree greater than any in use at the present time is presented. This type of container is particularly serviceable when removal of the plasma is contemplated for use in plasma transfusions.
2. Insofar as the changes in electrolytes are concerned, the large wide mouthed preserving jar is as effective a means as any in storing bloods for early use. It has, however, the disadvantages of being the easiest to contaminate, is not adaptable to the usual closed system of withdrawal and is unsuitable as a container in a plan which contemplates the removal of the plasma at some period after a week.
3. The small 500 cc. wide mouthed jar, filled to the very top is unsuitable.
4. The "Transfuso-vac" system is the easiest to use for

withdrawal of blood, freest from possible contamination and the simplest to use in giving a transfusion. The cell changes which go on, however, are more rapid and extend over the widest range of any of the containers tested so far. This raises the question of keeping cells, whose natural environment is one of considerable pressure, in a vacuum, also the question of trauma to the cells as they are squirted against the bottom of the flask with considerable force at the time of taking.

5. In a series of one hundred and thirty-one transfusions, the highest plasma potassium value observed, 236 milligrams per cent. This means that 0.7 grams of free plasma potassium were introduced into the patient who received that blood. It is equivalent to 0.014 gram per kilogram in a 50 kilogram man. If animal experiments give comparable results it would require roughly 2,500 cc. of blood at this stage of deterioration, given at a fairly rapid rate, to approach real toxic levels as a result of the potassium content.

6. The rate at which sodium decreases is approximately proportional to the rate at which the potassium increases. In the "dumbell" flask there was a difference of 26 milliequivalents between the highest and lowest potassium values and a difference of 22 milliequivalents between the highest and lowest sodium values in this loosely constructed clinical experiment.

There seems no place that the sodium in such quantities could have gone except into the cells, hence the rate of sodium loss becomes almost as accurate index of the change in red blood cell membrane as the potassium increase.

The answer to the problem of storing blood seems to lie in finding somemethod of maintaining the vital, selective properties of the cell membrane.

CHAPTER V

Part VIII

The Relation of Plasma Potassium Levels in Recipients Blood to Reactions

This series of experimental studies was begun because it was felt that in the high potassium content of blood which had been preserved over considerable periods of time, there might be found a part of the solution to some of the unexplained catastrophes and reactions incident to blood transfusions.

Procedure

Recorded here are observations on seventy-six patients. Bloods were obtained for analysis of their plasma potassium content in each case before the transfusion was given. In fifty-eight cases there was no reaction of any kind. In thirty-four, bloods were secured for potassium determinations from a vein of the arm opposite the one in which the transfusion was being given, just as the transfusion ended. In twenty-four cases, blood was collected for examination one hour after the transfusion ended.

In eighteen cases a reaction was noted within two hours of the end of the transfusion. Bloods were collected during the chill or during the time when the temperature was elevated in order to compare the levels before and after transfusion in these patients with the levels found in the patients who

had no reactions. The results are shown in table 48.

Table 48

Reactions in Relation to Plasma Potassium Content

Reaction	Number	Potassium as Milligrams Per Cent		
		Before	Immediately After	One Hour After
no	34	15.47	16.02	
no	24	16.37		16.9
(Average) no	58	15.8	16.3	
yes	18	16.5	16.4	
Total	76	16.0	16.5	

Discussion

In practically every case without reaction, there was a slight rise in the level after transfusion. In quite a number of those cases where there was a definite reaction, there was a slight fall.

There were no hemolytic reactions, none of marked severity.

Conclusion

There was no relation between the level of plasma potassium of seventy-six patients transfused with preserved blood and the occurrence of reactions.

CHAPTER V

Part IX

Summary of Clinical Observations and Recommendations

1. There is adequate response to transfusions of preserved blood as indicated by increases in red cell count, hemoglobin, cell volume, and protein content. When bloods have been stored for more than a week, there is a tendency for some of the increase to be lost in the following day.

   It is suggested, therefore, that in so far as the red cell quality is concerned, bloods may be stored a week, then used for transfusion with the expectancy of maximum response. After this time it is advised that the plasma be removed from the cells and stored in saline for future use.

2. The white cell response is so variable that no conclusions can be drawn. It has been established that leucocytes disintegrate rapidly and pari passu with their disappearance there is loss of hemobactericidal power.

   It is suggested, therefore, that when bloods are required because of their possible antibody content and immunological properties, that fresh blood be used or

at least blood stored for forty-eight hours or less.

3. While platelets do not disappear entirely and apparently do not reach ineffectual levels for a period of at least fifteen days, the number is greatly diminished.

   It is suggested, therefore, that patients suffering from diseases associated with thrombocytopenia be transfused with fresh or relatively fresh blood when possible.

4. The prothrombin content of preserved blood, while diminished, remains at effective levels as long as the blood is adjudged suitable by other criteria. It is, therefore, useful therapeutically in cases of hemorrhage due to prothrombin deficiency.

5. Blood stored over long periods gradually becomes more toxic as a result of the increase of potassium in the plasma. Certain contraindications have been outlined in chapter IV to insure safety from this potential source of trouble.

6. Blood taken in an atmosphere of carbon dioxide retains electrolyte balance more completely, therefore insures longer life and vitality of the cells. It would seem a wise procedure to collect all bloods for storage in

an atmosphere of carbon dioxide.

7. Glucose added to the preservative definitely prevents hemolysis, maintains the pH at a level near that of neutral and lengthens the life of the cells.

The best preservative at present would seem to be an isotonic citrate-saline-glucose solution.

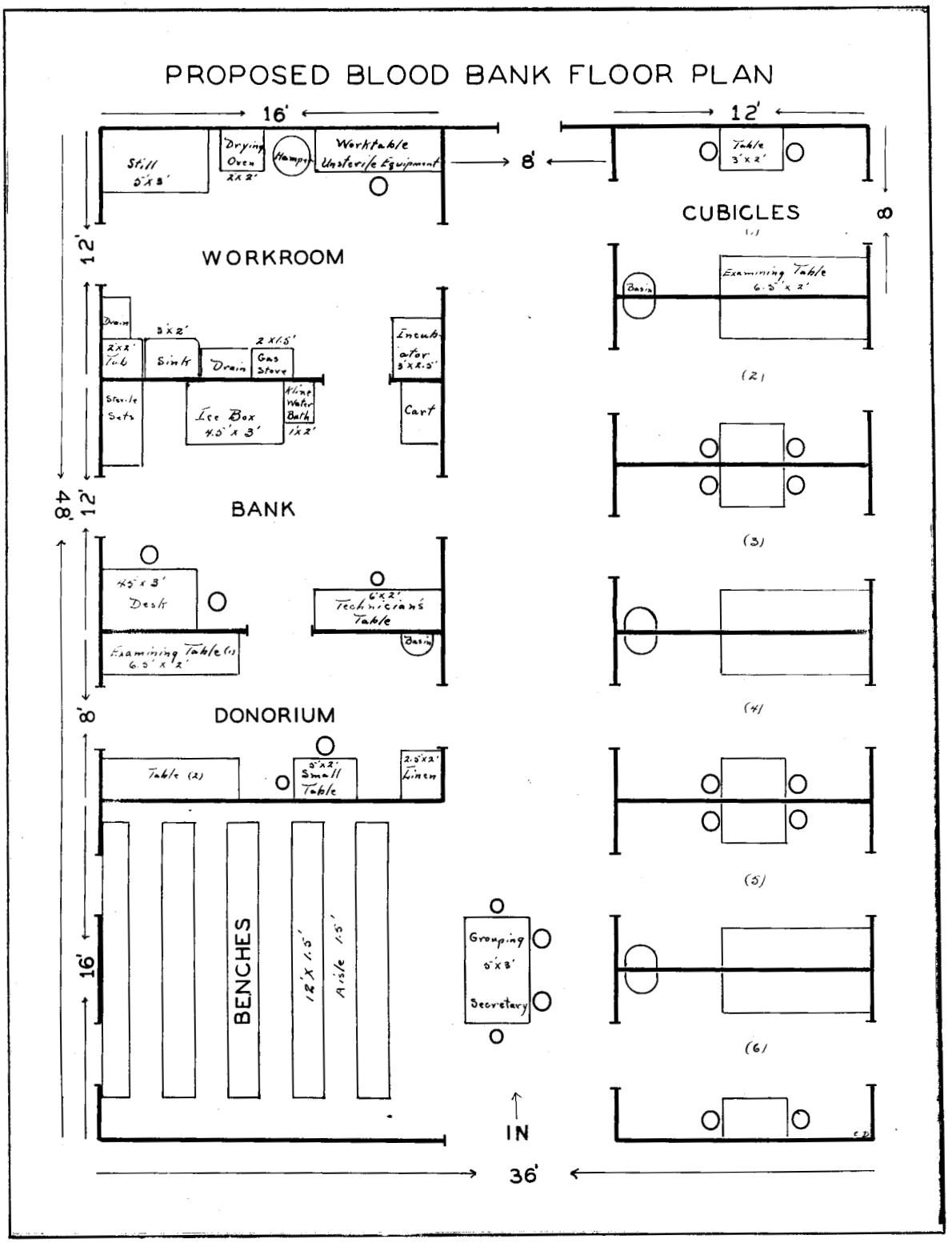
8. Bloods are kept better when the interface between the cells and the plasma is kept at a minimum. A flask has been designed to effect this improvement. It should be made a part of the routine equipment of the hospital.

9. Banked blood is safe when its limitations are known. The advantage of having ready for instant use bloods of all types is obvious.

The blood bank should become a part of the hospital service.

10. A blood bank to function well should have all of its activities centered in one place so that blood might be taken, tested, stored, and dispensed with the greatest ease, speed, and efficiency. A floor plan for such a center is appended.

FIGURE 21



This plan consists of two units. One contains the bank, workroom, and donorium and it should be centrally located in the hospital. The other, with six cubicles and benches, need not be immediately adjacent to the central unit but should be used for the "blood bank clinic" where large numbers of donors are handled.

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